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GROWTH OF THE HEN'S OVUM [1]

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PROBLEM

Considerable study has been made of the hen's ovum during development; however, the record of its growth is far from complete. Insofar as the writers have been able to determine the earlier observations were made from autopsies or after the feeding of the dye, Sudan III. Autopsies obviously provide a rather inaccurate method of measuring ovum growth, and the fact that the interval between feeding and actual deposition of the dye in the ova was not definitely known limited the value of such studies.

Stieve (*13*) [2] constructed theoretical growth curves for the hen's ova by removing and weighing all of the more mature ova of individual hens. Riddle (*8*), Romanoff (*11*), and Marza and Marza (*4*) have given some attention to the hen's developing ovum, and Bissonnette and Zujko (*1*) studied the development of the ovum of the starling. However, in most of the earlier studies emphasis was placed on stages of growth prior to those considered here and the technique utilized was not such as to give critical information of rate of growth. The period here considered is primarily the late stage of greatly accelerated growth which Riddle (*9*) described as starting when the ovum has a diameter of about 6 mm and having a growth rate as much as 25 times that of the previous period.

In the domestic hen ovulation occurs more or less regularly and continuously over long periods. Facts related to the growth of the ovum, therefore, have a bearing on the rate of ovulation and yolk size, which in turn influence the size of the fully formed egg. The physiological basis of differences in rate of laying and egg size was sought in this study.

STOCK AND METHODS

Most of the birds used in this study were White Leghorns, although a few Rhode Island Reds and first-generation hybrids were included. During the course of the experiment the hens were usually kept in individual hen batteries, where they were readily available for injection. The birds used were in their first or second year of production.

It was planned to study the growth of the hen's ovum by use of a fat-soluble dye such as that used by Riddle (*7*) and described more fully by Gage and Gage (*2*) and Rogers (*10*). The junior author suggested the possibility of injecting the dye directly into the blood stream of the bird, thus making possible more accurate time studies. In the studies of earlier workers the dye was fed.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 893.

A number of fat-soluble dyes were tested, but all were found less satisfactory than the Sudan III utilized by earlier workers. Very few of the fat-soluble dyes were deposited in the ovum. If deposited, they were so lacking in intensity as to give a poorly defined band of color. Sudan IV was nearly as satisfactory as Sudan III.

The dye was prepared for injection by forming a colloidal dispersion in water, by a method similar to that commonly used for substances soluble in alcohol but insoluble in water. A saturated solution of Sudan III in chloroform was first prepared, since the dye is much more soluble in chloroform than in alcohol. The chloroform solution was diluted with alcohol only as it was used, because the alcohol solution formed seemed to be supersaturated, and much of the dye crystallized out on standing. Approximately 4 cc of this solution was diluted with about 10 times its volume of alcohol, and this mixture was poured very slowly into 200 or 300 cc of boiling distilled water. After the chloroform and alcohol had boiled away, more dye was added until a satisfactory concentration was reached. After the last of the alcohol was boiled away, the red colloid was passed through filter paper while still hot. The concentration of dye was determined by diluting a sample with exactly 9 volumes of alcohol and comparing it on the colorimeter with a standard solution of Sudan III in 90-percent alcohol. It was intended that the dye should always be more concentrated than necessary at this point, so that after the concentration was determined, it might be diluted to the standard concentration, which in this case was 0.4 mg per cubic centimeter.

The usual injection was made with 2 mg of the dye in 5 cc of water. It was at first believed that adding daily this quantity of water to the blood stream would be harmful to the bird and thus interfere with egg production. However, the use of even greater quantities seemed in no way to harm the bird. Physiological salt solution was not used since it would have precipitated the dye.

In preliminary experiments to determine the most satisfactory dosage of dye, the quantity of Sudan III injected at a time was varied from 2 to 27 mg. The dosages 2, 4, 8, 14, and 27 mg gave bands of dyed yolk (in the same hen's egg) having mean widths of 0.39, 0.51, 0.63, 0.75, and 1.02 mm, respectively. Although the widths of the bands of dyed yolk did not vary proportionately, they did vary in the direction of the dosages.

Access to the blood stream was gained through the vena humeri profunda and its branches, a vein on the underside of the wing. This is the only vein which is large enough and exposed for sufficient length to meet the needs for repeated injections. By starting at the terminal branches of the vein and alternating between the two wings, as many as 16 daily injections could be made with ease. The chief difficulty in repeating the injections is the discoloration of the tissues due to hemorrhage, which makes the vein difficult to locate. A small nick was cut in the skin immediately over the vein, and a 1-inch (26-gage) needle was inserted in the vein. It was found that bending the needle at about a 30° angle greatly facilitated penetration of the vein. Two persons are required for the operation since the slightest move by the bird will tear out the needle and hemorrhage will obscure the vein for considerable distance. Straps attached to a table are fastened firmly about the bird's thorax and legs thus reducing the opportunity for struggling.

FIGURE 1.—Yolk secretion: *A*, Functional ovary showing gradations in size of immature ova. Above are two recently ruptured follicles. *B*, Transverse section of a boiled yolk showing distortion of inner dye bands. *C*, Note the sharp depression of the dye bands at the neck of the latebra. *D*, *E*, Two yolks showing dye bands resulting from injections at 24-hour intervals. Note the neck of the latebra showing as a break in the dye bands.

Injections were made at uniform intervals, usually 24 hours, the time of day depending on the nature of the experiment. The distance between color bands (fig. 1, *D*, *E*) was used as a basis of measuring growth rate. This technique made it possible not only to calculate the rate of growth at any point in the yolk but also to estimate accurately the size of the yolk at various periods previous to ovulation. In measuring growth the eggs from injected birds were boiled for 20 minutes; the yolks were weighed and halved at the germinal disk with a razor blade. A thin section was taken from the cut surface for measuring the distance between dye bands. A microscope with an eyepiece micrometer and mechanical stage was used for the measuring. The section of the yolk was placed on a slide and adjusted so that the zero reading was on the periphery. Measurements were then taken on the positions of all color bands in the yolk, recording the scale reading for the inner surface of each band. Readings were taken at two positions on the same section of the yolk, one opposite and one at a right angle from the germinal disk, so as to correct irregularities in the bands. The distances of the bands were expressed as the mean of the two measurements. The use of a drop of glycerin on the cut surface sometimes aided in differentiating the dye bands resulting from injections at 12-hour intervals. Readings must be taken immediately upon application of the glycerin since it rapidly disintegrates the yolk. The ovum growth for the interval of time under investigation was represented by the radius increment as measured from the inside surface of one band to the corresponding position on the succeeding band. The inside surface of the band was much more sharply defined than the outside and was a relatively accurate measure of yolk size at the time of injection.

In each injection series, measurements were started as soon as dye appeared in the laid eggs, usually the second day following the injection, and continued as long as the dye rings persisted. Thus eggs at the beginning of a series would have bands only on the outside of the yolk, and those at the close of the series would have them near the center only. Eggs at the middle of the series would have bands throughout. In most experiments, 11 daily injections were made.

In order to determine how soon the dye was deposited in the yolk, hens were killed at varying short intervals after injection, and the entire ovary was removed and boiled. In one hen, dye was unmistakably detected in a half-grown ovum which was removed 31 minutes after the injection. This was the minimum period in which dye was found, but several other tests showed periods closely approaching this. Since it probably requires a few minutes before enough dye is accumulated to be visible, it was assumed that deposition takes place immediately following injection.

YOLK SECRETION

The large number of egg yolks utilized in this study made it possible to make a number of observations on the structure of the ovum and its manner of growth. Diagrams of the hen's ovum as shown in most texts on embryology do not indicate very accurately the manner of yolk accumulation. If a section is made bisecting the blastoderm, the latebra, and its neck throughout the length in an egg carrying dye rings at frequent intervals, a more accurate picture may be gained of the manner of deposition.

Because of the length and narrowness of the neck of the latebra it is difficult to secure a section through its entire length. Such a section as diagrammed in figure 2 shows that the successive layers of yolk do not form a right angle with the neck but tend in the outer portions of the yolk to follow the lines of the neck itself for a short distance. Figure 2 is a diagram showing the daily deposition of yolk as measured in a transverse section of the ovum. Frequently the dyed areas of the ovum show the region of the yolk mass contiguous to the neck of the latebra to be rather sharply depressed toward the center of the ovum (fig. 1, *C*). There seems to be considerable variability in the degree of depression of the layers of yolk, at the neck of the latebra, some ova showing very little. However, this may be due to the position of the transverse section, since some show the distortion much better than others. Immature ova do not show the depressed lines near the neck of the latebra, indicating that the layers of yolk as originally secreted slope upward along the neck and that the distortion develops at some time after deposition.

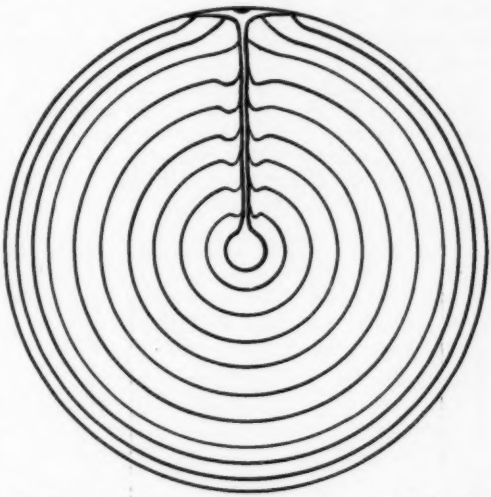

FIGURE 2.—Diagram of the yolk of an egg of hen No. 3925, giving the position of dye bands resulting from nine 24-hour-interval injections. Each concentric ring indicates the size of the yolk at the time of the injection. The latebra is in the center, and its neck joins the germinal disk.

Upon examining a yolk carrying dye rings throughout, one is impressed with the extreme uniformity of deposition. This fact made possible a study such as is here reported (fig. 1, *D*, *E*). The Regular spacing of dye rings secured by injections at uniform intervals shows the secretion of yolk to be both regular and uniform with respect to the surface of the ovum. It is also evident that there is very little flow or diffusion of materials within the yolk mass. Eggs kept for as long as 10 days had the dye bands as sharply defined as fresh eggs. This might be expected, since the dye is confined to the yolk globules and should diffuse very slowly through the water phase separating one globule from the next. The globules themselves, because of their size and close packing, would not be expected to move much except under pressure.

The uniformity of the layers of yolk leads one to speculate as to the nature of yolk secretion. Possible influencing factors are the composition of the blood stream in general, the vascular supply of the individual ova, and the permeability of the vitellin or follicular membranes. If the limiting factor were the surface over which secretion

might occur one would expect the radius increments to remain uniform. As will be shown, this is not the case since the augmentations of the radius decrease rapidly as the ovum matures.

The studies also have shown that the actual volume of yolk secretion increases throughout growth except during the last 3 days. If the blood supply is the controlling factor we must assume that this improves as the ovum matures. Any such improvement would be in the vascular system of the particular follicle. It could not be due to changes in the composition of blood itself since ova in all stages of growth are being developed simultaneously.

In some eggs an interesting phenomenon was observed in connection with the manner of yolk secretion. Frequently the innermost dye bands were badly distorted. Such a condition is shown in figure 1, *B*. The fact that such distortions were not found in the bands of immature ova observed in one phase of this study, would seem to indicate that the inner bands were not laid down irregularly. It is possible that the distortions of the bands of yolk near the center may have resulted from excessive yolk deposition at some point or points in the follicular wall. If this continued for some time it would cause irregular pressure on the interior of the yolk and probably would result in shifting within the yolk mass. In such ova it was impossible to secure measurements of growth.

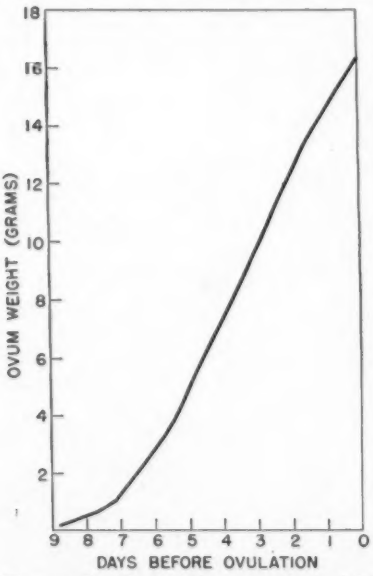

FIGURE 3.—Cumulative growth (weight in grams) curve constructed from the data on 367 yolks.

Observations indicated that although quantitatively yolk secretion was very uniform, there was evidence of qualitative variations. There were occasional conspicuous variations in yolk color. The most common qualitative difference appearing in the boiled yolks was a rubbery consistency of the interior instead of the characteristic granular nature. In the portion showing the rubbery consistency the dye bands were much diffused, indicating that the yolk material must have been much altered even before boiling. Riddle (*8*) mentions some yolks having a solid waxy interior; this is probably the condition found by the writers.

NORMAL GROWTH

The cumulative growth curve given in figure 3 is based on data from 38 birds, 367 eggs, and 1,169 dye bands. The records are from laying females of various ages, both White Leghorns and Rhode Island Reds. No significant growth differences were observed to be associated with age or breed.

The figure shows the growth in grams and time in days previous to ovulation. The actual measurements were recorded in units of the

eyepiece micrometer at points of known distance from the surface of the ovum. To express the results in grams, the ovum was assumed to be a sphere (which is not exactly true), and the radius was calculated from the weight and specific gravity of the ovum. Then the measurements were converted to distances from the center, and the daily volume increments in grams were calculated from the density of boiled yolk, which was determined to be 1.027. The zero point on the horizontal scale is the time of ovulation, and the highest point in the curve is the yolk weight of the laid egg.

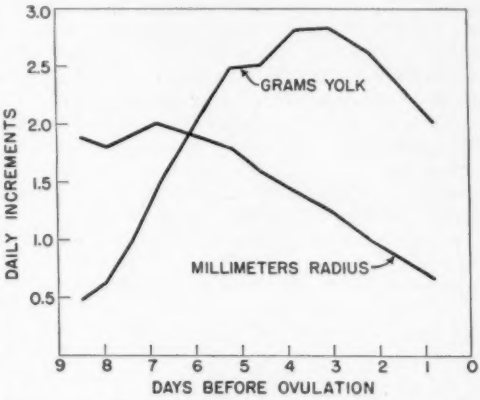

FIGURE 4.—Yolk growth curve showing the daily increments of radius and weight in 367 eggs.

It should be kept in mind that ovum growth as considered here includes only that period of relatively rapid increase in size occurring in the last 7 to 10 days before ovulation. Previous to that, growth is too slow to be measured by the technique here utilized. The curve indicates relatively slow yolk secretion for the first 2 days of the period (9 and 8 days before ovulation). Beginning the third day, the slope of the curve shows a higher rate of secretion, with evidence of a slight decline in the last 24 hours.

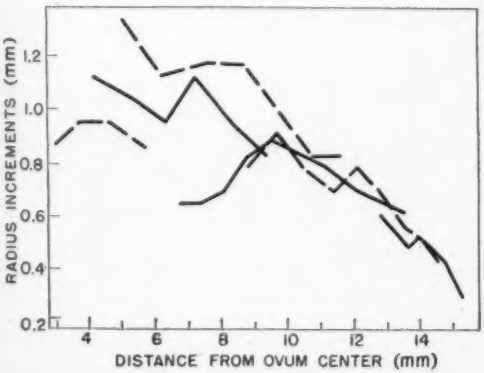

FIGURE 5.—Growth of individual ova of eggs laid by a single hen, measured in radius increments. The broken lines represent the growth of first eggs of a clutch and the unbroken lines that of successive eggs in a clutch.

A more definite picture of the rate of yolk secretion in the final cycle of rapid growth is shown in figure 4. Here growth is expressed as daily increments in grams of weight and millimeters of distance on the radius. It is seen from the figure that the growth when measured in units of the radius remains relatively constant until between 7 and 6 days before ovulation, when the ovum is about one-tenth its mature size. Then the daily radius augmentations are rapidly reduced.

It must be kept in mind that the surface of the ovum is increasing as it matures and even with constant increments of volume, the daily extension of the radius would decrease. For this reason a more enlightening measure of rate of yolk secretion is shown by the curve in figure 4, based on daily weight increments. This curve is of a form very different from that based on increments of the radius. The daily secretions of yolk material rapidly increase, reaching a somewhat flat maximum when the ovum has acquired about 60 percent of its size about 3 days before ovulation. During the last 48 hours there is a rapid decline in the rate of deposition of yolk material. So from the viewpoint of the amount of yolk materials secreted the efficiency of the follicle increases more or less progressively until the last few hours, when the rate of deposition declines rapidly.

Stieve (*13*) concluded from the examination of immature ova found in the hen's ovary that growth was very irrgeulár. He believed that there was frequent alternation between rapid and slow daily growth. Since the curves in figure 4 are the expression of the mean growth rate of many ova, such alternations of growth rate would be obscured.

In figure 5 are shown the curves of 12-hour period growth rates of six individual successive ova of a hen. The growth curves of the ova of this particular hen are typical of those of a number of birds from which such data were obtained. Since the dye was injected for a limited period, growth data were available for only parts of most eggs. There are some irregularities, but there is no alternation of slow and rapid growth such as was described by Stieve. This fact is also brought out in figure 6. Some of the irregularities probably are due to distortions of the dye bands. There is evidence in figure 5 of some variation in growth rate among the ova of a hen, but in general the curves are in close agreement. Exceptional growth rates of individual ova of a hen were frequently noted in this study, indicating that factors operating within the follicle may have some influence on growth.

EARLY GROWTH

The data presented in figures 3 and 4 were not satisfactory for measuring accurately the initial and the final stages of growth of the ovum. Since the dye injections were made at 24-hour intervals any changes occurring in the last few hours previous to ovulation would not be detected. Another rather serious source of error was irregularities in the innermost bands of dyed yolk. The cause of these distortions was not determined, but when they occurred they were more evident in the bands near the center. In order to secure more accurate data on the initial and final stages of ovum growth a special technique was utilized. The term early growth is used here to apply only to the early stages of the final period of rapid growth. Other workers have shown that the ovum grows for months or years, but studies here reported include only the final stage, where growth is rapid enough to be measured by means of dye injections.

The growth near the center of the ovum was studied by a series of four 12-hour-interval injections of hens that were killed immediately after the last injection. This method had the disadvantage of securing immature ova, the mature sizes of which could not be determined, but it did give ova in which the dye rings were practically free of distortions. Since the ova thus secured were of varying size the measurements reading from the surface of the ovum, were not com-

parable. To overcome this difficulty the radius of each ovum was calculated from the weight and specific gravity. Then all measurements were converted into distances from the calculated center of the ovum. Ten laying females were killed after four injections, and measurements were made of growth in 12-hour periods. The latebra of the ovum is largely the ovum as it existed previous to the initiation of the final cycle of growth. The exact outline of the latebra is not well defined since the white latebra materials fuse gradually into the outer yellow yolk. In a hard-boiled yolk the latebra is of a soft, mushy consistency. The radius of the latebra is roughly 3 mm so the growth detectable by dye injections should start at approximately that distance from the center of the ovum. The smallest measurable dye ring had a radius of about 2 mm. The data from immature ova indicate that the early measurable growth is quite uniform, being near 1 mm in 12 hours. For 12-hour growth periods measured at 2, 3, 4, 5, 6, 7, and 8 mm from the center of the ovum; the mean increase in the radius was 0.91, 0.97, 0.96, 0.98, 0.97 0.92, and 0.99 mm, respectively. Inasmuch as the measurements were in units of the radius and the size of the sphere was constantly increasing, uniform increments would mean that the weight augmentations were increasing. Calculations of the growth in weight for the five periods were 0.10, 0.16, 0.26, 0.39, and 0.50 g, respectively. These figures were based on rather small numbers, four to seven band widths at a point, but since the remainder of the curve, taken on the same number of samples, is normal in shape, it seems that the results probably are dependable.

In most instances the measurements of growth of yolks from laid eggs were not considered accurate near the center. However, after the available data were studied, 11 eggs were selected with dye bands near the center which showed none of the irregularities frequently found it bands at this region. The mean growth rate was for a 24-hour period and was calculated from five to seven bands at each point. The growth recorded at points 3, 4, 5, and 6 mm from the center of the ovum were 1.30, 1.98, 1.82, and 1.73 mm, respectively, as measured on the radius. The foregoing data agree closely with those from immature ova since the latter were recorded for a 12- rather than a 24-hour period.

These results indicate that the initial growth rate of the ovum (first 48 hours) as measured by units of the radius is relatively uniform. The fact that the initial radius increments remain approximately constant means that the actual amount of yolk secretion must be increasing.

LATE STAGES OF GROWTH

The growth in the last few hours previous to ovulation is of interest since in some animals it has been proposed that a last-minute acceleration of growth might be responsible for rupture of the follicular membranes and for ovulation. The observations of Phillips and Warren (*6*) did not support this view, but the technique of the present experiment makes it possible to secure more accurate information on the final stages of ovum growth.

Since the routine injections of dye were made at 24-hour intervals, the data in figures 3 and 4 were not of much value in determining the rate of growth in the last few hours previous to ovulation. It has been shown by Warren and Scott (*14*) that ovulation takes place on

an average one-half hour after the laying of the previous egg. Thus oviposition may be considered as a fairly accurate indication of ovulation time, but this holds only for eggs produced on successive days. Observation of a hen's laying record made it possible to predict that she would lay on the next 2 days. Such birds were injected with dye at from 1 to 15 hours previous to oviposition. The egg laid following injection was unaffected since it was in the oviduct at that time. However, since ovulation takes place almost immediately after oviposition of the previous egg, the period between injection and oviposition would measure the period of yolk deposition before ovulation. Since deposition of the dyed yolk starts immediately following injection, the distance between the inside of the dye ring and the surface of the egg would measure the amount of yolk materials secreted in the known period.

The question might be raised whether the use of dye injections measure the late-stage growth. If the final secretions were of a different nature they might fail to combine with the dye in the blood. However, observation indicated that this was not true since many eggs were found which carried a densely colored exterior.

Eighty-three hens were injected at varying short intervals previous to ovulation. When the egg resulting from ovulation following the injection was laid, measurements were taken of the growth of the ovum.

The data from this experiment are given in table 1 and are classified on the basis of the period of deposition. It should be kept in mind that the so-called period of deposition is the period intervening between injection with dye and the next oviposition. The ovulation which terminates deposition occurs at least a few minutes after oviposition so that the periods listed are somewhat less than those actually intervening. Inaccuracies of measurement are also a source of error of considerable consequence where very short periods of yolk deposition are involved. However, the results should not be vitiated as the comparison is of relative growth.

TABLE 1.—*Ovum growth in period immediately previous to ovulation*

Period of yolk deposition (hours)	Birds	Growth per hour		Period of yolk deposition (hours)	Birds	Growth per hour	
		Range	Mean			Range	Mean
	Number	*Millimeter*	*Millimeter*		*Number*	*Millimeter*	*Millimeter*
0.3 to 1.1	9		[1]	6.0 to 9.0	20	0.021–0.042	0.032
1.0 to 1.7	12		[2]	9.0 to 12.0	8	.034– .046	.038
1.0 to 3.0	4	0.015–0.036	0.025	12.0 to 15.0	17	.024– .057	.040
3.0 to 6.0	13	.013– .061	.032				

[1] No dye. [2] Trace.

In the eggs of nine females in which the period of yolk deposition ranged from 0.3 to 1.1 hours no dye could be detected. In 12 other females ovulating from 1.0 to 1.7 hours after injection the yolk showed such a slight trace of dye that it was not measurable. Four females having periods of deposition from 1 to 3 hours had measurable quantities of yolk deposited after the injection. In the egg of at least one female dye was detected when oviposition occurred just 1 hour after

injection. Previous work at this station would indicate that ovulation might be delayed for as much as an hour after oviposition, although the average period is probably much less. This would mean that at least some yolk is secreted in the last 1 or 2 hours previous to ovulation.

From the data in table 1 there is fairly consistent evidence that the radius augmentations gradually become less and less as the ovulation time is approached. The evidence for this view as shown in table 1 is minimized by the fact that the longer periods included both the slower and more rapid growth stages. That is, the 12- to 15-hour group does not show just the rate of deposition in the 3-hour period between 12 and 15 hours before ovulation but for the whole period of 12 to 15 hours. If the stage of later growth could have been segregated from the earlier growth the differences in table 1 would probably have been greater. Owing to the small number of measurements available and the extent to which differences in yolk size would influence the results, no attempt was made to calculate the amount of yolk secreted in the periods considered. There is no evidence of any acceleration of the rate of deposition in the period immediately previous to ovulation, and it is probable that the retardation of secretion, as indicated in figure 4, continues to the time of ovulation.

YOLK SIZE

One of the problems of interest in the initiation of this study was the relationship of ovum growth to egg size. Other investigations at this station have indicated the variations in egg size of an individual hen may be influenced by fluctuations in yolk or ovum size. Therefore the cause of such variations is to be sought in the process of ovum growth. Differences in yolk size might be due to either or both the factors, rate and period of growth. The available data are not well adapted to measuring accurately the variations in period of growth, but they are suitable for comparisons of rate of growth.

Rate of growth as related to yolk size was measured by daily increments of the radii of the yolks, at varying distances from the yolk surface. In figure 4 it is seen that the daily radius increments decrease rapidly as the surface of the yolk is approached. Thus a comparison of the radius increments at the same distances from the outside of small and large yolks introduces a source of error. Such a point might be one-fourth of the way through a small egg and only one-fifth the way through a large one, thus making the two points not strictly comparable because of the decreasing growth rate nearer the surface. This source of error probably is not great but it cannot be ignored. However, this appeared to be the only manner in which growth could be compared.

In order to avoid the influence of other factors the birds of each injection experiment were arbitrarily divided into large and small yolk-size groups for comparison of rate of growth (radius increments). Although this limited the size range somewhat it was believed that comparing only the large- and small-yolk birds of the same injection experiment eliminated many variables such as breed, age, and season. Five injection experiments each including from 8 to 11 birds were studied. Growth in millimeters of the radii of the two groups in each experiment was compared at seven points ranging from 1 to 14 mm from the surface of the yolk. There were from 10 to 54 measure-

ments for establishing a mean at each point in each size group. In the five-injection experiments 33 comparisons were made of the large- and small-yolk birds at various positions on the radius. In 18 of the 33 comparisons slightly slower growth was observed in the large-yolk groups. It appears probable from these studies that there is no difference in growth rate which may be considered a cause of yolk-size variation.

Since it is known that in the early period of a pullet's production her egg and yolk sizes rapidly increase, it is of interest to compare the growth rate of yolks of the same bird at different ages. Measurements were made of the growth rate of yolks of the very first eggs of eight White Leghorn pullets and again of yolks when the pullets were about 2 months older. In that period the mean yolk size had increased from 11.4 to 14.7 g, approximately a 29-percent increase. In table 2 is shown a comparison of growth rate of the yolks of these pullets at the two ages. Here again there was no consistent difference between the two sets of data. At three points the older birds had a slightly higher growth rate, and at six points the yolks of the younger birds grew more rapidly. If the 315 measurements of bands taken in the initial period are averaged they show a mean daily radius increment of 1.5 mm. The same birds when they were 2 months older and were laying larger eggs had a mean daily radius growth of 1.4 mm, calculated from 339 bands. The total number of measurements here do not agree with those in table 2 since some of those at extreme points were eliminated because the number of measurements was too small to give a dependable mean.

TABLE 2.—*Comparison of daily growth as measured on radius of the first eggs of a group of pullets with later and larger eggs of same individuals*

[Measurements were made at varying distances from the outside of the ovum]

Description of eggs	Distance from outside of ovum at which daily growth was measured	Measurements	Mean growth	Description of eggs	Distance from outside of ovum at which daily growth was measured	Measurements	Mean growth
	Millimeters	*Number*	*Millimeters*		*Millimeters*	*Number*	*Millimeters*
First pullet eggs (mean weight 11.4 g).	2	34	0.88	Later eggs of same pullets (mean weight 14.7 g).	2	47	0.97
	3	40	1.16		3	36	1.18
	4	32	1.33		4	32	1.36
	5	32	1.50		5	26	1.46
	6	28	1.61		6	27	1.61
	7	28	1.78		7	28	1.75
	8	19	1.87		8	28	1.65
	9	34	1.79		9	30	1.65
	10	28	1.70		10	24	1.69
	11	20	1.68		11	19	1.59
Mean			1.50	Mean			1.40

It would seem from these results that differences in yolk size among individuals and the yolks of a single bird are probably not the result of more rapid growth. The results tend to show that for a given

period the radius increments were the same in large and small yolks. Since it is admitted that large yolks have larger radii, the writers are forced to conclude that the growth must have continued for a longer period. It appears then that the data support only the view that some yolks are larger than others because they grow at the same rate for a longer period.

RATE OF LAYING

The data secured in this study should also throw some light on the physiological basis of rate of laying. It is known that differences in clutch (successive eggs) length are directly the result of interval variations (period between ovipositions). Frequency of ovulation varies with the frequency of oviposition. The question arises: Is the more frequent rate of ovulation due to a higher rate of deposition of yolk materials? In order to answer this question the coefficient of correlation was calculated for the hen's mean interval (period between ovipositions) and the mean radius increase of her eggs for a 48-hour period. The mean growth rate measured by radius increase was secured by averaging the growth at a point near the middle of the radius. This avoided discrepancies resulting from differences in growth rate at different positions on the radius.

The coefficient of correlation between rate of yolk secretion in the ovum and period of egg formation (interval) was based on 108 eggs and had a value of -0.21 ± 0.04. The coefficient is very low and of questionable significance. The minus value would indicate that the birds that lay more frequently have a slightly more rapid yolk growth, but the correlation value is so low that at best, rate of ovum growth can have only a minor influence on rate of laying. With a given amount of yolk-building materials in the blood stream, the amount of secretion in any one follicle would depend on the number of developing follicles drawing on the supply. Therefore, it seems quite plausible that no close agreement would be found between rate of ovum growth and rate of laying. That is, a bird lays more rapidly because of having more ova growing simultaneously rather than because of an increased rate of secretion in a fixed number of follicles.

CLUTCH POSITION

The relationship of clutch position of the egg to its growth is of interest. The laying of the first egg of the clutch is usually delayed 15 to 20 hours longer than the interval ordinarily intervening between successive eggs of a clutch. The interval between eggs is usually over 24 hours and results in the hen's laying a little later each day. When her schedule would cause a hen to lay late in the afternoon, she usually misses that day's production and starts a new clutch the next morning. Scott and Warren (*12*) have shown that the delay in oviposition of the first egg of the clutch is not to be accounted for by any additional time that the egg is in the oviduct but rather to a postponement of ovulation. This fact regarding the first egg of the clutch makes it of interest to compare the growth of the first and later eggs of a clutch with the view of gaining information on the mechanism responsible for the production of eggs in clutches.

In three experiments involving 26 birds and 270 eggs the measurements of growth were summarized to compare the first and later eggs

of the clutch. The data were sufficient to construct reliable curves, which are shown in figure 6. The curves were constructed on the basis of growth at varying points measured from the surface of the yolk. The data are not well adapted to check on the time of initiation of or the period of growth, but they do show that yolks of first and later eggs in a clutch have a very similar rate of growth and form of growth curve.

The segregation of egg laying into clutches resulting from a delay of ovulation of the first egg of a clutch might be due to a number of factors. One possibility is that a slower growth rate of the yolk of the first egg of the clutch delays the time of ovulation. Another possible explanation is that the yolk of the first egg of the clutch grows normally but that the initiation of the late stage of rapid growth is delayed. If this explanation were correct, an examination of immature ova of a laying hen at any time should show an irregular gradation of the first egg of the clutch as compared with the preceding and succeeding egg. If the beginning of the development of the yolk of the first egg of the clutch were delayed, then at all stages of growth the difference between its size and that of the preceding egg (the last egg of the foregoing clutch) should be greater than the difference between it and the yolk of the succeeding egg which is a member of its own clutch. It would not be possible to make such a comparison from autopsies since clutch position could not be identified, but from the dye-injection data such comparisons were readily available.

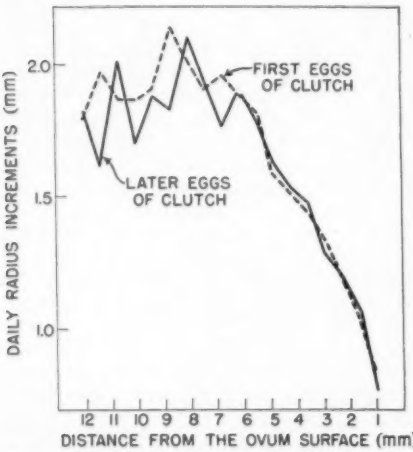
DAILY RADIUS INCREMENTS (mm)
2.0
1.5
1.0
FIRST EGGS OF CLUTCH
LATER EGGS OF CLUTCH
12 11 10 9 8 7 6 5 4 3 2 1
DISTANCE FROM THE OVUM SURFACE (mm)

FIGURE 6.—Comparison of mean growth curves of first and later eggs of a clutch. Radial growth was measured at different points beneath the yolk surface.

From a large series of eggs data were summarized as to the relative sizes at various stages of growth of the yolk of the first egg of a clutch, that of the egg which preceded it, and that of the one which followed it. In all the eggs the distance of each dye band from the outside of the ovum was used as a measure of its size. So for any injection, eggs in the before-mentioned clutch positions could be compared in a single hen. Since measurements were made from the outside of the ovum there would be some error because of differences in yolk size. However, since the fluctuations of yolk size of a single hen are small, this source of error would be slight.

The size differences between the yolks of the first eggs of the clutch and the preceding and succeeding eggs at varying stages of growth were tabulated, there being 208 such comparisons. Statistical treatment showed the mean difference in radii between the yolk of the first egg of a clutch and the yolk of the last egg of the previous clutch

to be 2.48 ± 0.04 mm, while the difference in radii between the yolk of the same first egg of a clutch and that of the second member of its own clutch was 2.48 ± 0.03. The results show that at all stages of growth there is maintained virtually the same size difference between the yolk of the first egg of the clutch and the last egg of the preceding clutch as there is between the first and second eggs of the same clutch. This would mean that the delay in ovulation of the first egg of a clutch is neither due to a delay in initiation of growth nor to a slower rate of growth.

This fact is further brought out in figure 7, which shows the cumulative growth curves of several eggs of the same hen. These curves are typical of a large body of data. As in figure 5 data were available for only parts of the curve for most ova. The slopes of the curves are very similar, indicating no differential growth rate between the ova of the first and later eggs of the clutch.

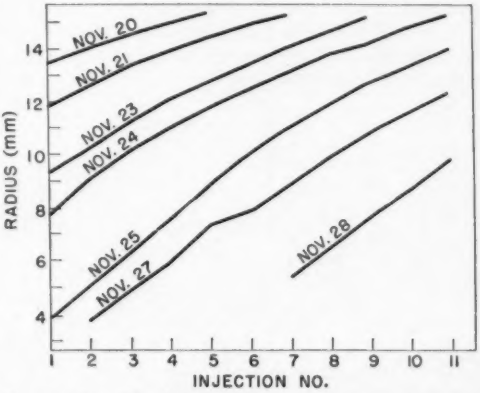

FIGURE 7.—Cumulative growth curves as indicated by radial length of individual ova of the eggs of a hen. The date of laying each egg is indicated on the curve. The points where the growth curves intersect the position of each injection indicate the relative growth rate of each ova of the series.

The distances between the curves of various ova are characteristically different throughout their growth. This indicates that not only did some ova start growing at a later period but that they maintained this difference throughout development. There was, however, no consistent relationship between these delays in the initiation of growth and clutch position.

It seems that the mechanism which segregates eggs into clutches does not act on the ovum until near the time of ovulation, and the effect is that the yolk of the first egg of a clutch has a longer total growth period than that of the other eggs. This difference would amount to only 6 to 10 hours out of the approximate total of 200 hours that constitutes the final period of accelerated growth. Since this is a rather small difference when compared with the total time and since the rate of growth decreases as ovulation approaches, when the difference in time appears, one would not expect a large difference in size between the first and later yolks of a clutch.

One of the interesting problems in connection with growth of the ovum is the relation of this phenomenon to light. Studies by Warren and Scott (*14*) showed that light is the major causative agent for the tendency of the fowl to produce eggs in clutches. This fact was established by modifying the period of light. It was not determined just how light had its effect, but changes in the light conditions were expressed in the laying reactions of the bird in about 50 to 70 hours

after the initiation of the change. This fact was arrived at from an experiment in which birds were subjected to a complete reversal of the periods of light and darkness. The birds responded by laying all of their eggs at night.

In one injection series the periods of daylight and darkness were reversed during the experiment for 14 White Leghorns. Thus ovum growth could be followed while the bird was adjusting itself to changed light conditions. It was hoped that the results might show how light acts normally in influencing the sequence of laying. The results were not very conclusive but seemed to show a retardation of about 10 percent in yolk secretion in the second 24-hour period after the change in lighting. The effects were greater in the less mature ova. Unless this result is due to the diminished food intake caused by the 24 hours of continuous darkness occurring at the time the change was made, no explanation presents itself.

YOLK FEEDING

Inasmuch as one of the factors controlling the rate of yolk formation must be the amount of yolk-forming materials carried in the blood, an experiment was conducted to determine the influence on ovum growth of feeding yolk material. Dye injections were made at 2 p. m. daily throughout the experiment. At the time of the fifth injection each of the 12 Rhode Island Red birds used was fed the yolks of two eggs and this was continued daily at the time of the six succeeding injections. Growth was measured in the yolks of all eggs produced as long as evidence of the dye injections was detectable in the eggs.

That the composition of the blood was profoundly affected by this feeding regime is shown by the fact that total fatty acids plus cholesterol in the blood was nearly trebled by the yolk feeding. Total fatty acids plus cholesterol was determined in whole blood by the method of Bloor as described by Hawk and Bergeim (*3, p. 442*). The average value for the group, which was 1,426 mg per 100 cc during the control period, rose to 3,964 during the period of yolk feeding.

TABLE 3.—*Ovum growth before and during the feeding of excessive amounts of yolk, the yolk feeding starting at the fifth dye-injection period*

Injection No.	Growth records	Mean radius increments in 24 hours following injection	Injection No.	Growth records	Mean radius increments in 24 hours following injection	Injection No.	Growth records	Mean radius increments in 24 hours following injection
	Number	*Millimeters*		*Number*	*Millimeters*		*Number*	*Millimeters*
1	43	1.69	5	37	1.68	8	34	1.81
2	40	1.57	6	31	1.71	9	33	1.84
3	39	1.60	7	33	1.84	10	34	1.76
4	35	1.77						

The growth from the time of one injection of dye to the time of the one which followed is given for the entire series in table 3. Growth was measured in millimeters of the radius. Each 24-hour growth period was measured by from 31 to 43 samples; adequate sampling from all parts of the ovum was thus provided, and the mean is

dependable. The ovum growth following the first four dye injections was made under normal feeding conditions, the birds receiving the standard Kansas State College laying ration. From the fifth injection on, the females received the two egg yolks per day in addition to the usual ration. It is not known exactly how much time would be required for the additional yolk feeding to have its effect on yolk deposition. It seems improbable that much effect could be noted in the 24 hours following the first feeding, and this period was disregarded in the comparison of growth rate before and after yolk feeding. The mean of the 157 24-hour radius increments previous to fat feeding was 1.67 $\pm$0.028 mm, while the 165 increments after the fat was fed averaged 1.83 $\pm$0.050 mm. Although the rate of growth was increased almost 10 percent in the period of yolk feeding, the difference is of doubtful statistical significance. In each 24-hour period after the sixth injection, growth was greater than the mean of the periods before yolk feeding.

It would appear therefore that additions of yolk fat to the ration may accelerate ovum growth. This stimulation might be expressed either in an earlier ovulation or a larger egg yolk. The weight of yolks of eggs laid during the first 4 days of the experiment were compared with those laid after the second day of fat feeding. The 29 yolks of eggs laid in the period before fat was fed had a mean weight of 17.75 $\pm$1.10 g while the 68 laid during the fat-feeding period weighed 18.29 $\pm$1.21 g. Although the yolks during yolk feeding were larger than those in eggs laid before, the increase was not enough to be considered statistically significant.

In order to determine whether the slight difference was really significant, a more extensive experiment was undertaken. Twenty-four White Leghorn pullets were used and were fed two yolks each for 10 days. The yolks of 98 eggs produced in the 5-day period just previous to yolk feeding were weighed individually. Beginning on the fifth day of yolk feeding, the yolks were again weighed for an 8-day period, in which 158 eggs were produced. Eggs were saved a little beyond the yolk-feeding period since yolk growth is completed at ovulation, which is 24 hours before laying and some time would be required for the assimilation of the additional fat that was fed. There is a possibility of a change in yolk size normally occurring in the birds during the period of the experiment as a result of age, temperature, or other uncontrolled factors. In order to eliminate errors from such a source, yolks were again weighed in a second control period following yolk feeding. Eggs were not saved until 6 days after the yolk feeding had been stopped. The control period following yolk feeding lasted 5 days; during that time 100 yolks were weighed. A recording thermometer was used, and the temperature during the yolk-feeding and during the two control periods was found not to vary appreciably.

The mean weights of the yolks produced during the yolk-feeding period was greater than that of those produced during either the preceding or succeeding control periods. The weight of the 158 yolks produced during the yolk-feeding period was 16.56$\pm$0.074 g, whereas the 98 yolks produced immediately before weighed 16.03$\pm$0.094 g. The 100 yolks produced in the control period following the yolk feeding weighed 16.21$\pm$0.107 g. If the results during the two control periods are combined, the mean yolk weight is 16.12$\pm$0.071 g. The

difference between the two control periods was not statistically significant, but the yolk weights during the yolk-feeding period were significantly greater than the weight of those produced in the control periods, the actual difference being more than four times the probable error of the difference. These results do not prove that the fat content of the blood is the limiting factor in yolk growth, but they indicate that rate of growth and yolk size may be increased by addition of yolk material to the diet.

KAMALA EFFECTS

Maw (*5*) has shown that the administration of kamala as a vermicide not only causes a decline in rate of production but also results in a decrease of egg size. Since variations in the whole egg size are frequently accompanied by changes in yolk size, kamala was fed to determine how it affected yolk growth. During the course of a dye-injection series 1 g of kamala was fed to each of four birds. Beginning about 24 hours after the administration of kamala, the yolk deposition not only was much retarded but it seemed to be of abnormal consistency. The dye rings were not sharply differentiated; this indicated a disruption of the secretory processes. It would, therefore, seem that kamala disturbs and checks the process of yolk secretion and so affects egg size.

SUMMARY

The technique of injection of the fat-soluble dye, Sudan III, into the hen's blood stream at uniform intervals was utilized for studies of growth of the ovum. Microscopic measurements of the daily radius increments from the concentric dye rings on the cut surface of boiled yolks made it possible to record the growth of the ovum. The period studied was the last 7 to 10 days before ovulation, in which growth is greatly accelerated.

When growth was measured in radius increments it was found to be relatively constant from the ninth to the seventh day before ovulation and then to decrease rapidly thereafter. If the actual daily secretion of yolk in grams is considered, it increases rapidly from the ninth to the fourth day before ovulation and after the third day before ovulation it decreases rapidly. Some growth was detectable within an hour of ovulation.

Yolk-size variations among hens and among an individual hen's eggs were not found to be associated with different growth rates of ova. This would indicate that variations in yolk size are to be accounted for by differences in the period of growth rather than by the rate of growth.

Differences in rate of laying as measured by the interval between ovipositions were not found to be closely associated with variations in rate of ovum growth.

The break between clutches (consecutive eggs) which is accompanied by a 15- to 18-hour delay in ovulation of the first egg of the clutch was studied in relation to ovum growth. No evidence was found of either a slower rate of growth or any delay in the initiation of growth. This would indicate that the first egg of the clutch may continue its growth longer than successive eggs of a clutch.

Feeding excessive amounts of egg yolk slightly increased both the rate of yolk secretion and the size of the mature ovum. Feeding kamala seemed to disrupt and retard yolk secretion.

The results of this study seem to indicate that variations in yolk size and the rate of ovulation are not conditioned by rate of ovum growth. The physiological basis of these phenomena is probably associated with the factors controlling the initiation of the final stage of accelerated growth.

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ADEQUACY OF LABELING OF CERTAIN TEXTILE FABRICS WITH REGARD TO FIBER CONTENT[1]

By HAZEL FLETCHER, *assistant professor in clothing and textiles*, and LOIS DENNHARDT, *research assistant, Kansas Agricultural Experiment station*[2]

INTRODUCTION

It has become difficult to ascertain the fiber content of fabrics by their appearance and handle or by chemical and burning tests. Formerly, it was a simple matter to determine whether a fabric was silk, cotton, linen, or rayon. New finishes, however, have been devised to give the synthetic fabrics the appearance of wool, linen, or silk. Furthermore, there has been an increasingly large production of fabrics composed of two or more kinds of fibers. Experienced textile workers have frequently been unable to determine fiber content without using exacting methods of identification. Because the kinds of fibers used cannot be determined without reliable laboratory techniques, fabrics on the market should have labels stating accurately the fiber content. Such accurate statements of the fiber content would enable the consumer to exercise proper care in dry cleaning and laundering and would be of particular value to those who are allergic to certain textiles (*18*).[3]

This paper reports an investigation to ascertain to what extent authentic information was available to the purchaser concerning the fiber content of fabrics on the open market.

RULINGS ON FIBER CONTENT OF TEXTILES

Some attempts have been made to state the fiber content of textiles. The Federal Trade Commission adopted a ruling on May 26, 1930, that "the word 'wool' shall not be used in any way in labeling, advertising, merchandising, or selling of knit underwear unless the percentage by weight of wool contained in the garment be stated."[4] A commercial standard for wool and part-wool blankets (*23*) became effective April 1, 1933, which provided for the labeling of part-wool blankets with the guaranteed minimum wool content, and one for wool and part-wool fabrics (*24*) became effective January 1, 1938. On October 27, 1937, the Federal Trade Commission issued its ruling of fair trade practices for the rayon industry (*20*). The new rules define rayon as "the generic term for manufactured textile fiber or yarn produced chemically from cellulose or with a cellulose base." In mixed fabrics containing rayon, it is required that the kinds of fibers and the percentages of each by weight be given on the labels. The ruling concerning rayon has met with some opposition. New rules were pro-

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[2] Contributions by the junior writer were based on results reported in a master's thesis, Adequacy of Labeling of Certain Mixed Fabrics. On file, library, Kans. State Col. Agr. and Appl. Sci.

[3] Italic numbers in parentheses refer to Literature Cited, p. 902.

[4] [UNITED STATES] FEDERAL TRADE COMMISSION. STATEMENT . . . Trade Pract. Conf., Knit Underwear Indus. July 7, 1930. [Mimeographed.]

mulgated November 4, 1938, for the silk industry. These rules recommend that silk fabrics be labeled as such and that mixed fabrics containing silk have the percentages of the different fibers given (*21*). The Federal Trade Commission also proposed trade practice rules for the wool industry in November 1938 (*22*).

MATERIALS

The fabrics tested in this investigation were purchased in 18 different stores in 7 towns and cities of the Midwest. At the time of purchase any information on the labels with regard to fiber content was noted. When the fiber content was not stated on the label, as much information as possible was obtained from the salesman. Of the 268 fabrics purchased, 133 contained only one kind of fiber, and 135 were of mixed fiber content. It was soon learned that the information concerning mixed fabrics and synthentic fabrics was least reliable, and an effort was made to select as wide a variety of these as possible. Few all-cotton and all-linen fabrics which were thought to be correctly labeled were purchased.

METHODS OF IDENTIFICATION AND ANALYSIS

MICROSCOPIC IDENTIFICATION OF FIBERS

Before any quantitative determinations of the fabrics were made, the kinds of fibers were determined. The microscope was generally used for identification because microdeterminations required less time and proved more conclusive than chemical tests. A comparison of longitudinal and cross-sectional mounts of the unknown fiber with a series of known specimens was employed. A permanent set of slides of authentic fibers most likely to occur in textiles was prepared. Plates 1 and 2 show photomicrographs of three rayons.

Many fibers could be identified by longitudinal mounts, but for the identification of silk and the various rayons cross sections were necessary. Cross-sectional mounts, accordingly, were made by the Viviani cork method (*5*, *14*, *15*, *25*).

Surface details of the wool fibers were studied by means of casts (*3*, *8*, *9*, *15*).

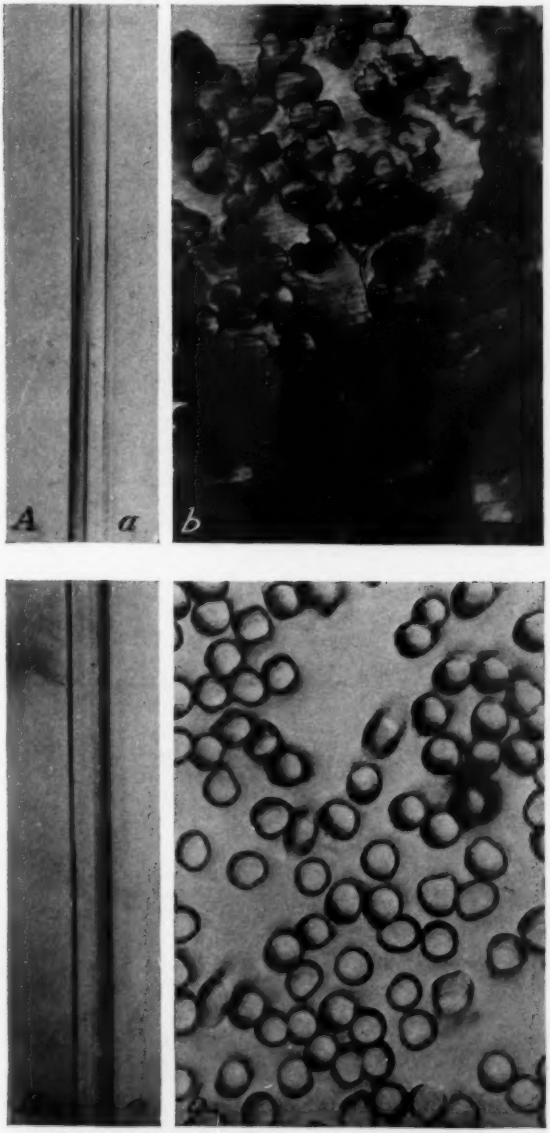
Polarized light proved to be valuable in the identification of highly birefringent vegetable and rayon fibers. Difficulty in differentiating between linen and mercerized cotton fibers was overcome by examination between crossed Nicol prisms. Polarized light brought the nodes of linen into greater prominence.

In some of the identification work, confirmatory tests were made by using certain dyes. Millon's reagent was used in distinguishing animal from vegetable fibers, and Neocarmin B and Meocarmin W were used in identifying the rayons.

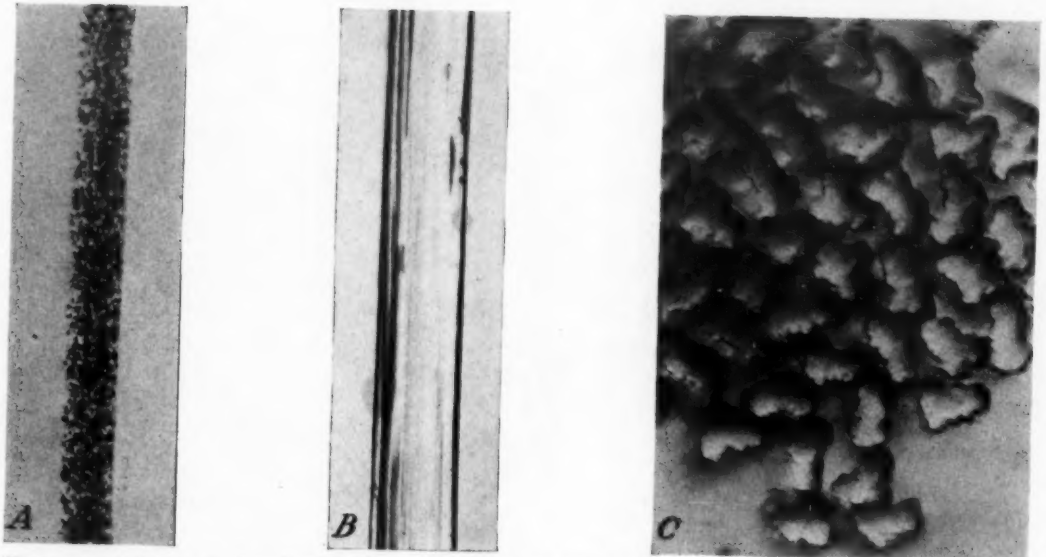
QUANTITATIVE MECHANICAL ANALYSIS OF MIXED FABRICS

When a fabric was composed of one kind of fiber, only the fiber identification was needed. When a fabric contained two or more kinds of fibers, a quantitative analysis was necessary. This analysis was made as follows:

Two samples of each fabric, weighing approximately 5 g were taken. The sizing, finishing, and other nonfibrous materials were



A, Cellulose acetate rayon fibers: *a*, Longitudinal view, Canada balsam mount, × 380; *b*, cross section, air mount, × 350. *B*, Cuprammonium rayon fibers: *a*, Longitudinal view; *b*, cross section. Glycerin mounts, × 380.



Viscose rayon fibers: *A*, Longitudinal view, delustered, Canada balsam mount, × 350; *B*, longitudinal view, lustrous, glycerin mount, × 380; *C*, cross section, lustrous, glycerin mount, × 380.

removed by extraction with carbon tetrachloride and a 3- to 5-percent aqueous solution of a starch-hydrolyzing enzyme. These operations were carried out according to the directions of the American Society for Testing Materials (*1*). The dried weight of the samples was found, and a suitable quantitative analysis was used in determining the fiber content.

There are three fundamental ways of determining the fiber content quantitatively—mechanically, microscopically, and chemically. The mechanical method when applicable is the most accurate. When the fibers are mixed before the yarn is spun, mechanical separation is not feasible. Ply yarns having one ply of one kind of fiber and one of another are often used in weaving fabrics. The mechanical separation of the plies is frequently a better method to use than a tedious and less accurate chemical analysis. Fabrics woven from two different kinds of yarn are easily analyzed mechanically. Many fabrics have the warp yarns of one fiber and the filling of another.

QUANTITATIVE CHEMICAL ANALYSIS OF MIXED FABRICS

Several chemical analyses were checked; samples of known fiber were used in order to determine which methods were most applicable. If cellulose acetate rayon was present in a mixed fabric, it was removed first by dissolution in acetone. Acetone readily dissolved acetate rayon with an error of less than 1 percent whenever cotton, silk, wool, or regenerated cellulose was present. The method followed in the use of acetone was that given in the Standards on Textile Materials (*1*). Two samples were run simultaneously and agitated at room temperature in the Launder-Ometer for 15 minutes in about 50 times their weight in acetone.

Silk, if present, was removed next. The method of dissolving silk in Lowe's reagent [5] at room temperature for 20 to 30 minutes was found to be the quickest and most accurate of those tried. The residual samples always gained weight because of the deposit of a gelatinous copper compound which was removed by rinsing the samples in a 1- to 2-percent hydrochloric acid solution before washing with distilled water.

There are several methods (*1, 2, 6, 7, 10, 12, 13, 19, 24*) of determining the wool content in mixtures of wool, cotton, and rayon. Cotton and regenerated cellulose may be separated from wool by carbonization with aluminum chloride. In the work reported herein, the sample was immersed for 10 minutes in a boiling solution of aluminum chloride containing 9 g of hydrated chloride per 100 ml of water. After the sample was heated in an oven at 105° to 110° C. for 2 hours, it was rubbed against a 100-mesh screen with sufficient pressure to powder the carbonized cellulose and pass it through the screen. The material was passed through the screen again to recover any wool fibers that had passed through the first time. The wool was agitated with about 100 ml of a 10-percent solution of hydrochloric acid, then washed with distilled water until free from chlorides and dried. The error involved in this method was less than 1 percent.

[5] The reagent is made of 16 g of copper sulphate in 150 ml of water, with the addition of 10 g of glycerol. A concentrated solution of sodium hydroxide is added until the precipitate of copper hydrate, which at first forms, dissolves.

When wool comprised the greater portion in a mixture of wool and cotton or wool and regenerated cellulose rayon, it was removed with a solution of potassium hydroxide as shown by Mease and Jessup (*12*). The samples were boiled for 10 minutes in a 5-percent aqueous solution of potassium hydroxide. A reflux condenser was used to prevent concentration of the alkali because of evaporation. The samples were rinsed in a 5-percent aqueous solution of acetic acid, and then in distilled water until free from acetic acid. Potassium hydroxide dissolved wool readily with little effect on cotton. Regenerated cellulose was affected somewhat by this method, the error being approximately 3 percent; but with cotton the error was less than 1 percent.

Cotton was separated from regenerated cellulose by an aqueous solution of calcium thiocyanate as described by the American Society for Testing Materials (*1*). The samples were agitated for 1 hour at 70° C. in 200 ml of calcium thiocyanate, of specific gravity 1.35 to 1.36, slightly acidulated with acetic acid. The error was approximately 2 percent.

Table 1 shows the effects of the various reagents upon the textile fibers. Correction factors were not used except when potassium hydroxide was used to dissolve wool mixed with regenerated cellulose rayon. The weight of regenerated cellulose was found by multiplying the dry weight obtained in the analysis by 1.03.

TABLE 1.—*The effect of various solvents on different textile fibers*

Fiber	Acetone	Lowe's reagent	Aluminum chloride	Potassium hydroxide	Calcium thiocyanate
Cellulose acetate.	All dissolved				
Silk	Less than 1 percent dissolved.	All dissolved			
Cotton	do	Less than 1 percent dissolved.	All carbonized	Less than 1 percent dissolved.	Approximately 2 percent dissolved.
Regenerated cellulose.	do	do	do	Approximately 3 percent dissolved.	All dissolved.
Wool	do	do	Less than 1 percent carbonized.	All dissolved	Approximately 2 percent dissolved.

QUANTITATIVE MICROSCOPICAL ANALYSIS OF MIXED FABRICS

Although there is an increasing realization that the volume of each fiber present in a mixed fabric is as important as the weight of each fiber present, comparatively little work has been done on quantitative microscopical analysis. Heretofore, most quantitative analyses of fibers have been based on percentage weight as determined by chemical analysis. The microscope lends itself to volumetric as well as gravimetric determinations. Quantitative microscopical analysis of textile fabrics is often the most desirable, and sometimes the only, method which can be used. Fabrics made of yarns which are blends of fibers lend themselves to microscopical analysis. If one kind of fiber constitutes a very small percentage, it is often better to resort to microscopical analysis rather than to a chemical analysis in which the loss of the fiber of small percentage may amount to a great deal.

Blends of viscose rayon and linen, or of viscose rayon and cotton may be analyzed by dissolving the rayon in calcium thiocyanate. After the solution is used, it must be diluted until the rayon is precipitated, and then evaporated before it is ready to be used again. The microscopical analysis is often to be preferred to the chemical analysis.

Blends of different kinds of wools cannot be separated by chemical analysis, and microscopical methods must be used. Skinkle (*16, 17*) and von Bergen (*26*) made analyses of wool and mohair and other animal fibers, identifying the kind of fiber by measuring scale sizes and diameters and counting the number of each kind in a cross section of yarn.

Heermann and Herzog (*4*) showed that the percentage of each kind of fiber in a mixture could be found by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a g_a}{n_a g_a + n_b g_b}$$

where n is the number of fibers and g is the weight of each fiber per unit of length. Skinkle (*17*) in his work on animal fibers, which are nearly round in cross section and of approximately the same density, expressed the percentage by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a d_a^2}{n_a d_a^2 + n_b d_b^2}$$

where d is the average diameter of the fibers and n is the number of fibers.

This equation can be modified to calculate the percentage of fibers which are of irregular cross section without the determination of weight of the fibers per unit length as done by Heermann and Herzog.

Two methods were used by the present writers for counting the number of each kind of fiber. For blends of linen and viscose rayon, of cotton and viscose rayon, or of cotton and linen, the number of each kind of fiber was counted by cutting as small a length of the yarn as possible, less than one-sixteenth of an inch, with small shears. The small lengths were mounted in glycerin and teased out as uniformly as possible on the slide. A magnification of 100 or less was used with a 16-mm objective, and the total number of fibers of each on the entire slide was counted by moving the slide with a mechanical stage which had two graduated scales at right angles. The distinguishing of linen from cotton could often be facilitated by placing the slide between crossed Nicol prisms. Ten slides or more were prepared from yarns taken from various parts of the fabric, and the average of the counts on the 10 slides was taken as the number of fibers of each kind in the yarn.

The above method was used for blends in which one kind of fiber made up a very small percentage of the yarn and for fibers of which it is difficult to make cross sections.

For blends of different kinds of wools, of rayon and wool, and of wool and cotton, the counting of the fibers was done by making cross

sections by the Viviani cork method. A magnification of about 300 with a 4-mm objective was used. A field was chosen at random, and the number of sections of each kind of fiber was counted. Ten to twenty different fields of the same size were counted, and the average of each kind was taken as the number of fibers. Plate 3 shows photomicrographs of cross sections of yarns of various kinds of mixed fabrics.

The relative areas of the fibers were found by drawing 10 or more cross sections with the camera lucida as pointed out by Preston (*14*). The cross sections were cut out and weighed on an analytical balance, and the average weight of the paper drawings of each kind of fiber was calculated and taken as proportional to the size of the fiber. The percentage by volume of each kind of fiber in a blend can be expressed by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a w_a}{n_a w_a + n_b w_b}.$$

The percentage by weight of each can be expressed by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a w_a g_a}{n_a w_a g_a + n_b w_b g_b}.$$

In the equations n is the number of fibers, w is the average weight of camera-lucida drawings of the cross sections, and g is the specific gravity of the fiber. The values of the specific gravities were taken from Heermann and Herzog (*4*) and the International Critical Tables (*11*).

RESULTS AND DISCUSSION

Table 2 gives the results of the examination of the 133 fabrics of one kind of fiber. In each case the information on the label and that given by the clerk were compared with determinations made in the laboratory. Ordinarily the salesmen gave no further information if the fiber content of the textile was stated on the label. An acetate rayon fabric was considered accurately labeled if given as celanese rayon, acetate rayon, or acetate, and only partially accurate if given as rayon or celanese. The information with regard to fiber content of a fabric of cuprammonium rayon was considered accurate if given as cuprammonium rayon, cuprammonium, or Bemberg rayon, and partially accurate if given as Bemberg or rayon. In the case of viscose rayon, viscose rayon or viscose was counted as accurate, and rayon as partially accurate.

A mixed fabric was considered to be accurately labeled if the kinds of fibers that it contained were given. Viscose rayon had to be given as viscose rayon or viscose; cuprammonium rayon as cuprammonium rayon, cuprammonium, or Bemberg rayon; and acetate rayon as acetate rayon, acetate, or celanese rayon. If the percentage of fiber content was stated, the information was considered accurate if the percentage was correct within 10 percent. It was considered partially accurate if one kind of fiber contained in the fabric was given, and the others were not. Table 2 also gives the results of the examination of these fabrics.

A, Blend of wool and cotton, cross section, glycerin mount, × 380; *B*, blend of wool, delustered viscose, and dyed viscose, cross section, glycerin mount, × 380; blend of wool, silk, and rabbit fur, cross section, glycerin mount, × 380.

TABLE 2.—*Adequacy of information secured from labels or salesmen concerning fabrics composed of one kind of fiber or of mixed fibers*

FABRICS OF ONE KIND OF FIBER

Fabrics analyzed		Fabrics that were—				Fabrics regarding which salesmen gave—			
Kind	Number	Labeled	Labeled accurately	Labeled partly accurately	Labeled inaccurately	Information	Accurate information	Partly accurate information	Inaccurate information
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Cotton	4	2	2	0	0	2	1	1	0
Linen	3	3	3	0	0	0	0	0	0
Silk	26	11	11	0	0	16	13	2	1
Wool	14	5	5	0	0	9	8	0	1
Acetate rayon	16	7	1	5	1	9	2	7	0
Cuprammonium rayon	13	5	1	4	0	8	1	7	0
Viscose rayon	57	24	0	23	1	35	0	31	4
Total	133	57	23	32	2	79	25	48	6
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Proportion		100	40.4	56.1	3.5	100	31.6	60.8	7.6

FABRICS OF TWO OR MORE KINDS OF FIBERS

		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Cotton and wool	11	2	0	2	0	11	4	5	2
Cotton and viscose	15	3	0	3	0	13	0	11	2
Cotton and linen	6	5	0	5	0	2	0	2	0
Wool and rabbit fur	3	0	0	0	0	3	1	2	0
Wool and viscose	21	4	0	4	0	19	0	18	1
Wool and silk	5	2	0	2	0	5	1	4	0
Silk and viscose	7	2	0	2	0	5	0	5	0
Acetate and viscose	43	12	0	12	0	31	0	30	1
Various fabrics composed of 2 kinds of fibers	6	1	0	1	0	5	2	3	0
Various fabrics composed of 3 kinds of fibers	18	3	0	3	0	16	1	14	1
Total	135	34	0	34	0	110	9	94	7
		Percent		*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
Proportion		100		100		100	8.2	85.4	6.4

SUMMARY

Table 2 shows that of the fabrics analyzed those made of cotton, linen, silk, and wool were commonly accurately labeled and that salesmen, in the main, gave accurate information with regard to these fabrics bearing no label with regard to fiber content. Of all the fabrics made of one kind of fiber, the information concerning the three types of rayon was found to be least accurate. These fabrics were often represented as "rayon," but they were usually not designated as acetate, viscose, or cuprammonium rayon. The word "celanese" was often used instead of acetate; Bemberg and Bemberg rayon were always used instead of cuprammonium rayon; and rayon was used instead of viscose rayon. Of the 133 fabrics of 1 kind of fiber analyzed, 57 were labeled. Of these, 40.4 percent were accurate; of the 79 for which salesmen gave information, 31.6 percent were accurate. Thus the information on the label was found to be more accurate than that given by the salesmen.

Of the 135 mixed fabrics, 117 were made of 2 kinds of fibers, and 18 were of 3 kinds. Many of these had warp yarns of one kind and filling of another. Some were made of yarns which were blends, and

others of ply yarns composed of plies of different kinds of fibers. Cellulose acetate rayon and viscose rayon were most frequently found in combination. Only one fabric of each of the following combinations was found: Viscose and linen, linen and wool, acetate and cuprammonium rayons, acetate rayon and silk, wool and cuprammonium rayon, and wool and mohair.

Little information was obtained concerning the percentage of fiber content, either from the labels or from the salesmen. Only one fabric bore a label stating the percentage of fiber present. The label stated that the fabric contained 25 percent of wool, but analysis showed only 15 percent to be present. The clerks gave percentages for fiber content for 12 fabrics, of which 1 was accurate, 10 partially accurate, and 1 wrong.

Of the 135 mixed fabrics examined, only 34 were labeled; and all of these were only partially accurate. The clerks gave information concerning 110, of which 8.2 percent were accurate, 85.4 percent partially accurate, and 6.4 percent wrong.

It is apparent that more of the fabrics of one fiber are labeled, and more of the information accurate, than of mixed fabrics, but in case of the labeled fabrics of one kind of fiber the accuracy was only 40.4 percent. The accuracy of the information, from labels and from salesmen, for both groups of fabrics was inadequate.

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A PHYSIOLOGICAL STUDY OF THE PREFILLING PERIOD OF FRUIT DEVELOPMENT IN THE PECAN[1]

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INTRODUCTION

In a report of the study of fruit development in the pecan (*Carya pecan* Engl. and Graebn.[2]) made in 1932,[3] the authors pointed out that lack of material prevented the collection of samples as frequently as was desired during the prefilling period. In a similar study made during the spring and summer of 1933 this condition was corrected by taking samples of Burkett nuts at least every 2 weeks from May 18 (the first sampling date in 1932) to August 28. Since the 1933 crop developed a little earlier than that of 1932, the nuts of the first samples were somewhat larger, and those sampled on August 28 were considerably further advanced than those for corresponding samples taken in 1932.

SAMPLING AND METHODS

During the first part of the experiment samples were taken every 2 weeks, but beginning August 1 they were taken each week in order to follow more closely the rather rapid process of shell formation. On August 14 and thereafter, separations were made between shuck and shell and samples of the two parts were preserved for analysis. The stages of development of the nuts on the various sampling dates are given in table 1.

TABLE 1.—*Stage of development of pecan fruits on the dates of sampling*

Sampling date	Weeks after full bloom	Average green weight per fruit	Stage of development
1933	*Number*	*Grams*	
May 18	6	0.22	All fruits apparently alike except that they increased in size as the season advanced.
June 1	8	.55	
June 15	10	1.54	
June 29	12	3.04	
July 14	14	6.89	
Aug. 1	16	16.53	Shell just beginning to harden at blossom end. Ovule had grown quite a distance into the white spongy material. Seed coat filled with clear colorless fluid or endosperm.
Aug. 7	17	19.85	Shell quite hard from apex to about half way to basal end of nut. Considerable layer of white spongy material between seed coat and shell. Ovule filled with clear colorless fluid of low viscosity.
Aug. 14	18	23.29	All of the shell sufficiently hard to permit removal of shuck by paring and scraping. Still some white material between seed coat and shell. Endosperm same as before.
Aug. 21	19	27.68	Whole shell quite hard. Cotyledons beginning to line seed coat. Endosperm more viscous than before. Spongy material still white except near bundles, where it was light brown.
Aug. 28	20	28.25	Cotyledons beginning to thicken. Endosperm mostly absorbed.

[1] Received for publication October 24, 1938.

[2] Synonym, *Hicoria pecan* (Marsh.) Britton.

[3] THOR, CLIFFORD J. B., and SMITH, CHARLES L. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. Jour. Agr. Research 50: 97-121, illus. 1935.

The sampling and analytical methods used were essentially the same as those employed for the 1932 material.[4]

ANALYTICAL DATA

The analytical data are summarized in table 2.

TABLE 2.—*Composition of pecan fruits throughout the prefilling period, 1933*

Part of fruit	Date of sampling	Constituents of dry matter					Weight per fruit or part					Ratio of total sugar to moisture
		Dry matter	Total nitrogen	Ash	Total sugar	Acid-hydrolyzable polysaccharides	Dry matter	Total nitrogen	Ash	Total sugar	Acid-hydrolyzable polysaccharides	
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Gram*	*Gram*	*Gram*	*Grams*	
Whole fruit	May 18	31.66	1.76	6.95	6.09	9.80	0.0701	0.0012	0.0049	0.0042	0.0069	0.0277
Do	June 1	29.95	1.53	6.29	7.09	9.86	.1662	.0025	.0105	.0118	.0164	.0303
Do	June 15	29.50	1.35	5.70	10.35	10.82	.4547	.0061	.0259	.0470	.0492	.0432
Do	June 29	29.54	1.23	5.70	10.64	10.97	.8980	.0110	.0512	.0956	.0985	.0446
Do	July 14	27.99	1.15	4.83	14.57	10.86	1.9294	.0222	.0932	.2811	.2095	.0566
Do	Aug. 1	22.09	1.08	4.09	16.02	10.11	3.6515	.0394	.1493	.5850	.3692	.0454
Do	Aug. 7	22.95	1.00	3.86	14.82	11.24	4.5567	.0456	.1759	.6754	.5122	.0441
Do	Aug. 14	24.39	.96	3.29	11.05	13.26	5.6804	.0545	.1869	.6277	.7532	.0357
Do	Aug. 21	25.91	.95	3.43	9.51	13.91	7.1719	.0681	.2460	.6821	.9976	.0333
Do	Aug. 28	28.60	1.01	3.89	8.26	13.81	8.0795	.0816	.3143	.6674	1.1158	.0331
Shuck	Aug. 14	26.51	1.29	4.87	12.03	12.86	2.5688	.0331	.1251	.3090	.3303	.0434
Do	Aug. 21	23.99	1.47	5.47	12.67	12.23	3.1460	.0462	.1721	.3986	.3848	.0400
Do	Aug. 28	22.56	1.31	6.45	13.07	12.10	3.3759	.0442	.2177	.4413	.4087	.0381
Shell plus kernel	Aug. 14	23.15	.65	2.28	9.22	14.62	3.0188	.0196	.0688	.2783	.4413	.0278
Do	Aug. 21	28.47	.65	2.26	6.64	15.70	4.0632	.0264	.0918	.2698	.6379	.0264
Do	Aug. 28	36.60	.72	2.00	5.32	14.08	4.7990	.0346	.0960	.2074	.6757	.0249
Kernel	Aug. 28	19.02	2.37	4.24	4.34	7.25	.9013	.0214	.0382	.0392	.0653	.0102

While the data for 1933 show the same general trends as those for the few samples taken during the same period of fruit development in 1932, they also show fluctuations in concentrations of various constituents during the prefilling period that were not apparent in the previous data. For this reason the data for dry matter, ash, total sugars, and acid-hydrolyzable polysaccharides are presented graphically in figures 1 to 4. The data for nitrogen are not shown graphically because the amount of this constituent increased steadily throughout the prefilling period, as it did in 1932.

The percentage of dry matter in the whole fruit dropped rather suddenly in July, reaching a minimum on August 1. During this same period there was a rapid increase in the actual amount of dry matter per fruit, caused by the rapid increase in size. The percentage of dry matter in the whole fruit increased from August 1 to the end of the experiment, owing to the development of the shell and shuck. The data indicate that during August the shell development was somewhat greater than that of the shuck, since the dry matter in the shell plus kernel increased more rapidly than that in the shucks. The hardening of the shell occurred during the first 3 weeks of August. The kernel contained relatively little dry matter at the end of August, and not all of this was developed during the month. From the middle to the last of August the percentage of dry matter in the shuck de-

[4] THOR, CLIFFORD J. B., and SMITH, CHARLES L. See footnote 3.

creased rapidly while the total amount per shuck increased. During this same period both the percentage and the amount of dry matter in the whole fruit and of the shell-plus-kernel samples increased. Thus, the whole fruit had its highest concentration of moisture on August 1, while the moisture concentration in the shuck increased rapidly during the latter part of August. In 1932 the percentage of moisture in the shucks increased through September, after which it decreased to the date of harvest.

A comparison of the changes in dry matter with rainfall records does not show consistent correlations. There was a sharp drop in the percentage of dry matter in the whole fruit from July 14 to August 1.

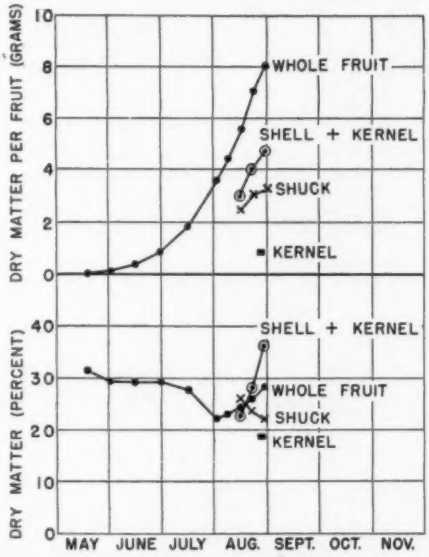

FIGURE 1.—Changes in dry-matter content of the pecan nut during the prefilling period.

1933, during which time the rainfall was 6.98 inches. However, there was also an appreciable decrease in the percentage of dry matter in the whole fruit from June 29 to July 14, yet no appreciable amount of rain fell from June 1 to the latter date. The rainfall from August 1 to August 28 was only 0.32 inch, yet the percentage of dry matter in the shucks decreased from 26.51 percent to 22.56 percent from August 14 to August 28.

The percentage of ash in whole fruits decreased regularly and rather rapidly to August 14, after which it started to increase, but the amount of ash per fruit showed a regular increase throughout the experiment. In the shuck the ash increased rapidly during the latter part of August both in amount and in percentage of the dry weight. In the shell-plus-kernel samples there was a small increase in amount of ash but a decrease in its percentage on the dry-weight basis. The increase in the percentage of ash in the whole fruit from August 14 to 28 was due largely to the rapid increase of ash in the shuck (fig. 2).

The changes in total sugar during the prefilling period are shown in figure 3. The percentage of sugars on the dry-weight basis increased rapidly in the whole fruit until August 1 and then decreased rapidly to the end of the experiment, but the actual amount of sugar increased regularly to August 7, after which it remained practically constant. The sugar in the shuck increased rapidly from August 14 to 28, while that in the shell-plus-kernel samples was decreasing. The drop in percentage of sugar in whole fruits was due to the rapid accumulation of dry matter of the shell and shuck. Since the amount of

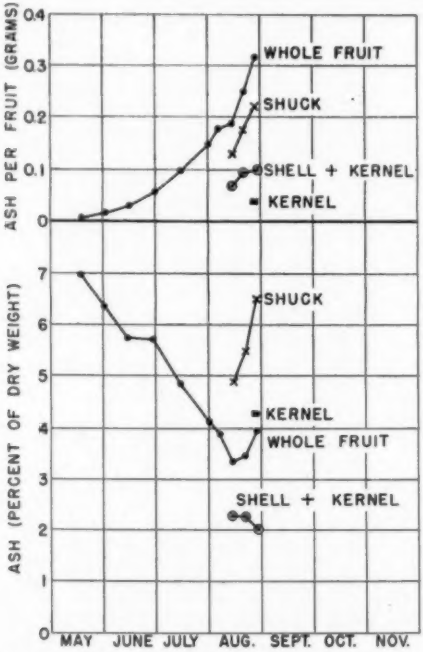

FIGURE 2.—Changes in ash content of the pecan nut during the prefilling period.

sugar in the whole fruit changed but slightly in August, an amount of sugar equivalent to the increased carbohydrate material of the shell and shuck must have been translocated from the tree to the fruit during the 3 weeks when the shell was hardening. The decrease in the sugar content in the shell-plus-kernel samples in late August may have been the result of its transformation to acid-hydrolyzable polysaccharides, which increased materially at this time.

The ratio of total sugar to moisture in the whole fruit rose rapidly from May to the middle of July and then dropped equally rapidly, the maximum occurring just a little before the first visible signs of shell hardening. The occurrence of this sharp maximum in sugar concentration is probably related to the process of shell development rather than to the beginning of division of the fertilized egg as was suggested in the previous paper.

The concentration of acid-hydrolyzable polysaccharides on a dry-weight basis increased slightly during the rapid period of growth of the fruit, then dropped a little, and with the beginning of shell hardening early in August rose sharply again to concentrations considerably above previous levels. This is what would be expected, since pecan shells have been found to contain a relatively large amount of acid-hydrolyzable polysaccharides yielding chiefly *d*-xylose on hydrolysis.[5] The drop in concentration the latter part of July was due to the increased water content of the shuck during that time. On a

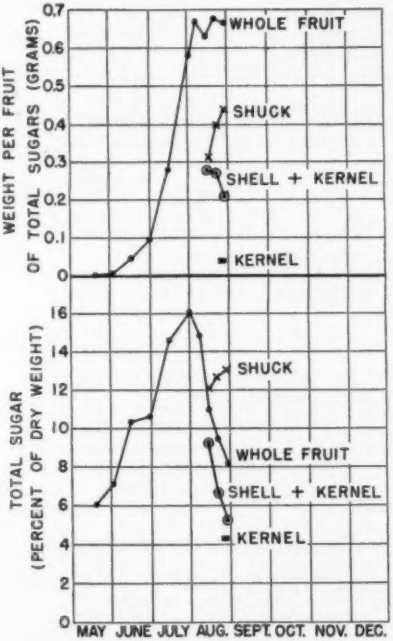

FIGURE 3.—Changes in total sugar content of the pecan nut during the prefilling period.

weight-per-fruit basis the acid-hydrolyzable polysaccharides in the whole fruit simply increased in a regular manner throughout the entire prefilling period, as was indicated by the 1932 data. The data show that this increase was due largely to the increase of these substances in the shell and to a lesser extent to their increase in the shucks. The kernels contained very little acid-hydrolyzable polysaccharides on August 28.

SUMMARY

The data presented for 1933 confirm the results for changes in composition of pecan fruits during the prefilling period of 1932. It is shown that the development of the shell is completed during this

[5] THOR, CLIFFORD J. B., and SMITH, C. L. PECAN SHELLS AS A SOURCE OF D-XYLOSE. Jour. Amer. Chem. Soc. 56: 1640. 1934.

period, since a comparison of these data with those for later dates in 1932 shows relatively little change in the amounts of constituents after the filling process begins, except a loss of total sugars. The shell developed rapidly during August, showing complete hardening during the first 3 weeks of that month. This development was accompanied by a rapid increase in acid-hydrolyzable polysaccharides and dry matter and a decrease in total sugars. The nitrogen simply increased steadily with the development of the nuts.

During the latter part of August there were rapid increases in total sugars and ash in the shuck, with lesser increases in dry matter and

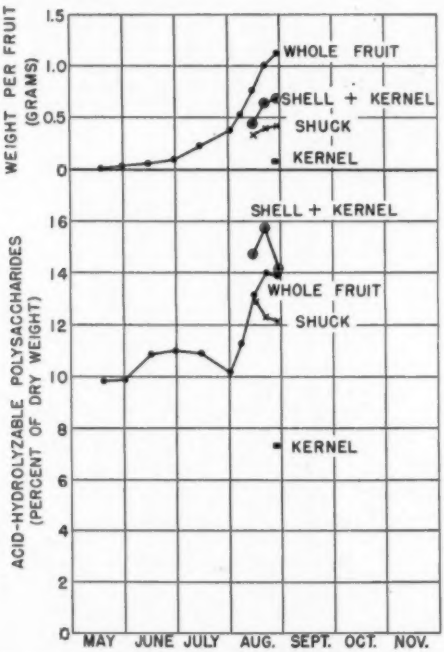

FIGURE 4.—Changes in acid-hydrolyzable polysaccharide content of the pecan nut during the prefilling period.

acid-hydrolyzable polysaccharides. By comparing the data for the 1933 season with those for later dates in 1932, it is shown that the shuck development was completed during the prefilling period, except for an increase in the ash content and slight changes in the total sugars and acid-hydrolyzable polysaccharides. The percentage of moisture in the shuck increased for some time after nut filling began in 1932, and was increasing at the beginning of this process in 1933.

While the present data from frequent sampling show the same general trends as data from the few samples for the corresponding period in 1932, they also show the fluctuations in amounts of the constituents in the nut during the prefilling period.

DWARFING OF CUCURBITS SPRAYED WITH BORDEAUX MIXTURE[1]

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INTRODUCTION

Bordeaux mixture has long been recommended as a protective spray for cucurbits to discourage the cucumber beetle and diseases. Many farmers have been loath to use the material, however, reporting that the treatment is more deleterious to the plants than the pests are. Copper injury was long suspected. This suspected injury was combated by reducing the copper or increasing the lime content of the mixture. Still farmers have persisted in using other materials or no fungicides at all on their cucurbits.

Bordeaux injury on plants has been under investigation at this station for several years. The two most important elements in bordeaux injury are the lime (*6*) [3] and the copper (*9*). Papers on the effect of bordeaux mixture on transpiration (*3*) and bordeaux injury on tomatoes (*4*) have already been published. Wilson (*10*) has investigated bordeaux injury to cucurbits.

There are six distinct symptoms of bordeaux injury on cucurbits: (1) Dwarfing of the plants; (2) distortion of leaf margins, (3) yellowing of leaf margins followed by necrosis; (4) scorching of leaf lamina; (5) accelerated transpiration; and (6) defloration. This paper deals with the dwarfing and leaf-deformation aspects of the problem.

Since bordeaux mixture is prepared from three components, copper sulphate, lime, and water, the effect of these three substances was investigated first. Their effect was not clearly evident, however, until the effect of spray load and hydrogen-ion concentration was understood.

MATERIALS AND METHODS

The first problem that presented itself was to measure accurately the deleterious effects observed in the field. Field trials were ruled out for exploratory work because striped cucumber beetles proved so serious on the plants on wnich no insecticides were used that they vitiated conclusions. It seemed best to do as much of the work as possible in the greenhouse away from the disturbing influence of the beetles. The greenhouse work was conducted during the winter seasons of 1936 to 1938 inclusive, and some of the conclusions were checked during the summer seasons of the same years.

Chicago Pickling cucumbers or Honey Rock musk melons were planted in rows 6 mches apart and 4 feet long in greenhouse benches filled with electrically pasteurized soil (*2*) to keep damping-off at a

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[2] The writers are grateful to R. O. Magie and George Pearce for assistance. This research project was conducted in cooperation with the Crop Protection Institute.

[3] Italic numbers in parentheses refer to Literature Cited, p. 926.

minimum. Between crops the soil was treated with formalin (*1*). Seeds were sown about 1 inch apart at a strictly uniform depth so that

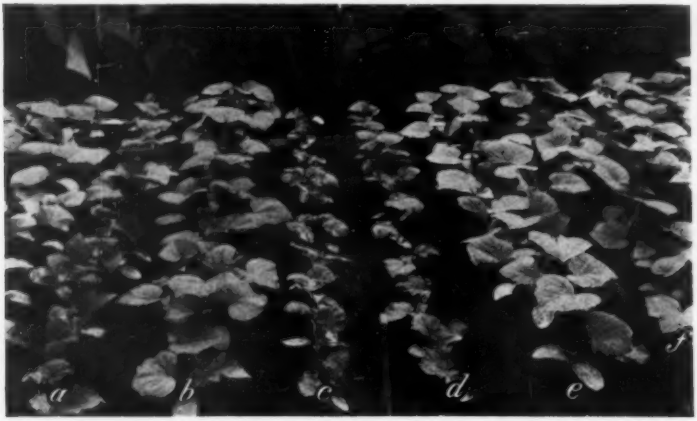

FIGURE 1.—Dwarfing of cucumber plants by various sprays (series 4a, table 1): *a*, Copper silicate; *b*, red cuprous oxide; *c*, copper silicate plus Bancroft clay; *d*, red cuprous oxide plus Bancroft clay; *e*, Bancroft clay alone; *f*, nonsprayed.

a uniform emergence of seedlings was obtained. Sprays were applied with a 1-quart hand atomizer. Treatments were applied on single

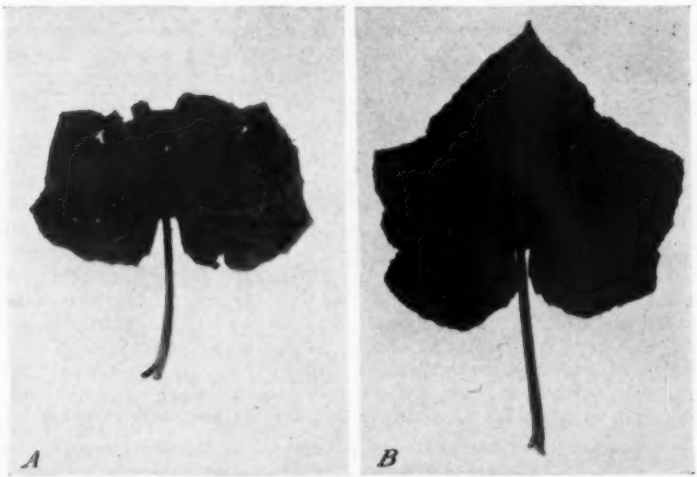

FIGURE 2.—Leaf deformation of cucumber caused by bordeaux mixture: *A*, Sprayed leaf; *B*, nonsprayed leaf.

rows of about 40 plants replicated from four to six times. Drift was prevented by baffle boards on either side of each row. Sprays were applied approximately weekly; i. e., when seedlings emerged, when

the first true leaf appeared, and when the second true leaf appeared. A photograph of a typical test appears in figure 1.

The basic spray was 4–4–50 or 5–5–50 [4] bordeaux prepared with hydrated lime. The word "lime" in the text refers to commercial or laboratory-made hydrated lime in all cases. All sprays were made up in tap water. The other materials were applied in equivalent copper concentrations or in equivalent spray loads. Plants used as checks were sprayed with tap water.

Plants were thinned at intervals by clipping them at the ground line and weighing them while green. From one to three harvests were made from each experiment. The separate harvests are referred to as "series" in the tables and text. By conducting the cultural operations carefully and by using pasteurized soil to obviate variations due to damping-off of seedlings, this technique proved adaptable, simple, and reproducible for measuring dwarfing and leaf deformation.

RESULTS

Dwarfing of cucumber plants by various sprays is illustrated in figure 1. The leaf-deformation symptom is shown in figure 2 in comparison with a normal leaf. Both dwarfing and leaf deformation are closely allied. They seem to be produced by the same factors. Data obtained in a greenhouse study of some of the factors that influence dwarfing of cucurbits are given in table 1.

EFFECT OF COPPER

It is very difficult to obtain an adequate evaluation of the copper factor in bordeaux injury. The use of copper sulphate alone is hardly practicable except as an indication, because the copper is so soluble as to be very toxic, producing necrosis and stunting which are far in excess of that produced by bordeaux and which are not typical of bordeaux injury.

The effect of the copper factor has been studied (1) by using insoluble copper compounds with and without lime, and (2) by varying the copper content of bordeaux and keeping the lime constant.

TABLE 1.—*Effect of various sprays* [1] *on growth of cucurbits* [2] *in the greenhouse*

[Data expressed as average green weight per plant in grams]

Materials used in spray	Weight per plant for date of harvest and series No. indicated								
	Mar. 2, 1936, No. 1	Mar. 9, 1936, No. 1a	Mar. 13, 1936, No. 1b	Mar. 9, 1936, No. 2	Mar. 14, 1936, No. 2a	Apr. 8, 1936, No. 3	Apr. 15, 1936, No. 3a	Apr. 22, 1936, No. 3b	May 4, 1936, No. 4
Water	0.684	1.61	2.60	1.48	2.65	1.33	2.65	5.76	0.87
Bordeaux	.488	1.49	2.02	1.19	1.74	1.15	2.05	4.15	.75
Red cuprous oxide	.605	1.73	2.67	1.49	2.39	1.33	2.46	5.29	[3].85
Red+Bancroft clay									.63
Copper silicate (Coposil)	.520	1.29	1.75	1.27	1.90	1.31	2.34	4.74	.71
Coposil + Bancroft clay									.61
Lime [5]	.582	1.51	2.06	1.51	2.27	1.36	2.67	5.39	
Lime [7]						1.25	2.15	4.52	
Bancroft clay									.81

See footnotes at end of table.

[4] Spray formulas are employed to simplify the text. A formula 4–4–50 bordeaux means 4 pounds of copper sulphate pentahydrate, 4 pounds of hydrated lime, and 50 gallons of water; and 1–50 of any material means 1 pound of the material in 50 gallons of water.

TABLE 1.—*Effect of various sprays on growth of cucurbits in the greenhouse*—Continued

[Data expressed as average green weight per plant in grams]

Materials used in spray	Weight per plant for date of harvest and series No. indicated							
	May 12, 1936, No. 4a	May 28, 1936, No. 5	Apr. 15, 1937, No. 6	Apr. 24, 1937, No. 6a	Apr. 15, 1937, No. 7	Apr. 24, 1937, No. 7a	May 13, 1937, No. 8	June 5, 1937, No. 9
Water	4.13	1.73	1.94	7.32	1.82	6.18	4.85	13.65
Bordeaux	3.25		1.69	5.47	1.51	4.11	3.45	11.24
Red cuprous oxide	[3] 3.98	1.65	1.89	6.87			4.37	13.09
Red+calcium arsenate							3.91	12.93
Red+lead arsenate								10.37
Red+lime		1.52					4.06	13.07
Red+lime+Bancroft clay							4.19	11.86
Red+Bancroft clay	1.93	1.32			1.54	4.20	3.68	11.34
Red+sulphur		1.71						
Red+diatomaceous earth		1.69						
Red+flour		1.69			1.83	6.14		12.21
Red+talc					1.64	5.93	4.24	
Red+Wyogel							4.17	
Red+gypsum							4.44	13.30
Red+Celite							4.44	
Red+Hammill & Gillispie clay							4.01	12.60
Red+magnesium oxide [4]							4.08	12.38
Copper oxychloride [5]			1.91	6.59				
Copper+talc					1.90	5.44		
Copper+clay					1.62	4.68		
Copper+flour					1.79	5.89		
Copper silicate (Coposil)	2.85		1.84	6.34				
Coposil+Bancroft clay	1.77							
Coposil+bentonite			1.82	6.30				
Coposil for citrus			1.91	5.99				
Lime [6]							3.65	12.34
Lime [7]							3.24	11.12
Bancroft clay	3.46		1.78	6.01				
Talc			1.86	6.44				
Wheat flour			1.88	6.37				
Derris			1.73	6.16				
Calcium arsenate			1.70	5.92			3.98	12.69
Lead arsenate								11.39

[1] Sprays were made up at the rate of 1.25 pounds of copper (as metallic) in 50 gallons of water except series Nos. 6 to 9, where the rate was 1 pound. Other materials, such as lime, etc., were added at the rate of 5 pounds in 50 gallons, except series Nos. 6 to 9, where 4 pounds was used.

[2] All data were obtained with cucumbers, except series Nos. 3, 3a, and 3b, in which muskmelons were used.

[3] Red cuprous oxide, 5 to 50 gallons.

[4] Magnesium oxide, only 4 ounces to 50 gallons.

[5] Grasselli A copper oxychloride.

[6] Lime equivalent to lime content of bordeaux.

[7] Lime equivalent to spray load of bordeaux.

The so-called insoluble copper compounds like red cuprous oxide (electrolytic grade, Cuprocide) or copper oxychloride (Grasselli compound A) did not seriously stunt cucumber foliage in the greenhouse (table 1), nor did they cause leaf deformation. Copper silicate (Coposil), however, was found to stunt plants considerably in almost every one of the 12 series in which it was included (table 1). Plants sprayed with copper silicate were invariably smaller than those sprayed with red cuprous oxide or copper oxychloride. The reasons for this are not clear, but the effect may have been owing as discussed below, to some diluent in the copper silicate and not to the copper.

Three experiments were made in which the effect of copper in bordeaux was measured by varying the copper content and keeping the lime constant (table 2). In the first two tests commercial hydrated lime was used. The degree of carbonation of this lime was not determined although one sample obtained from stock the same winter showed 38 percent of carbonate. Lime for the third test was burned

and slaked. Observation of the curves (fig. 3) for the three experiments indicates two possible conclusions (1) that copper has little or no effect, or (2) that this type of test does not demonstrate it very clearly. The bimodal shape of the curves in two of the tests indicates that factors other than the copper were chiefly responsible for their shape. As the copper content of the spray increased, the spray load increased and the pH value decreased. The effect of these factors on the shape of the curve is discussed below.

TABLE 2.—*Effect of hydrogen-ion concentration and spray load of various mixtures on growth of cucumbers*

Material	Spray formula [1]	Spray load, quantity to 50 gallons	pH	Green weight per plant		
				May 1937 [2]	June 1937 [3]	February 1938
		Pounds		*Grams*	*Grams*	*Grams*
Water		0	7.5	4.85	13.65	1.660
Bordeaux	4-1-50	5.0	4.4	3.72	11.10	1.378
	4-2-50	6.0	11.7	3.94	11.93	1.460
	4-4-50	8.0	12.3	3.45	11.24	1.317
	4-6-50	10.0	12.3	3.44	11.19	1.159
	4-8-50	12.0	12.3	3.43	10.73	1.088
	1-4-50	5.0	12.3	4.04	12.08	1.247
	2-4-50	6.0	12.3	3.73	11.22	1.276
	6-4-50	10.0	12.1	3.36	11.11	1.408
	8-4-50	12.0	11.7	3.75	11.22	1.471
Cu_2O	1.2-50	1.2	6.40	4.37	13.09	----------
Cu_2O+Bancroft clay	1.2-5-50	6.2	3.56	3.68	11.34	----------
Cu_2O+Hammil & Gilispie clay	1.2-5-50	6.2	4.65	4.01	12.60	----------
Cu_2O+Celite	1.2-5-50	6.2	7.55	4.44	----------	----------
Cu_2O+gypsum	1.2-5-50	6.2	8.66	4.44	13.30	----------
Cu_2O+talc	1.2-5-50	6.2	8.88	4.24	12.69	----------
Cu_2O+magnesium oxide	1.2-.25-50	2.0	9.70	4.08	12.38	----------
Cu_2O+calcium arsenate	1.2-5-50	6.2	9.70	3.91	12.93	----------
Cu_2O+Wyogel	1.2-5-50	6.2	10.49	4.17	----------	----------
Cu_2O+lime	1.2-5-50	6.2	12.30	4.06	13.07	----------
Cu_2O+lime+clay	1.2-2.5-2.5-50	6.2	12.30	4.19	11.86	----------
Lime	4-50	4.0	12.30	3.65	12.34	----------
	8-50	8.0	12.30	3.29	11.12	----------

[1] The numbers in the formula refer to the quantity of the ingredients in pounds per 50 gallons; the first figure shows copper, the second, lime for bordeaux or diluent for Cu_2O.
[2] This is series 8, table 1.
[3] This is series 9, table 1.

These data indicate that insolubilized copper is not a primary cause of dwarfing and leaf deformation on cucumber plants. The copper in bordeaux is insolubilized with lime in excess. It was important, then, to investigate the effect of the excess lime.

EFFECT OF LIME

When the lime content of a spray, in which only lime was used, was made equivalent to the lime content of bordeaux mixture, the plants were dwarfed and the leaves deformed, but the effect was not so great as that of bordeaux (table 1). If the content was increased so as to equal the spray load of the bordeaux the stunting increased, but still was not quite so serious as when bordeaux was used. Leaf deformation also increased with an increase in lime.

The effect of the lime was studied also by varying its concentration in bordeaux and keeping the copper content constant (table 2 and fig. 4). The curves from data in three tests (fig. 4) all show that, generally speaking, growth fell off as lime content of the bordeaux increased.

The only exception was that growth did not fall off as the lime was increased from 4–1–50 to 4–2–50. This increase in growth with increase in lime was not caused by a direct beneficial effect of the lime

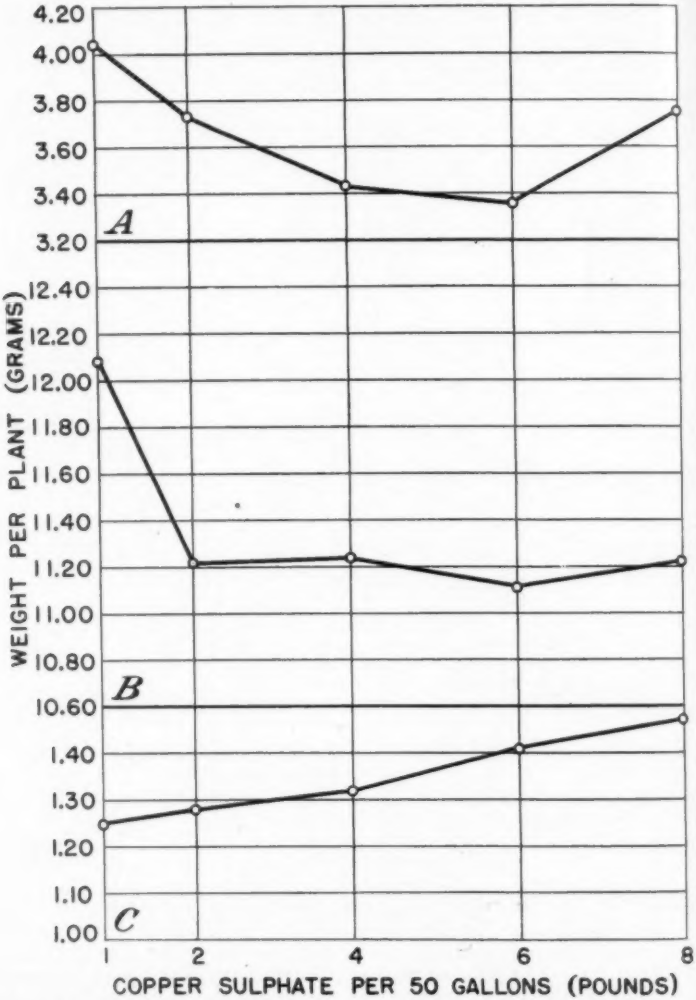

FIGURE 3.—Effect of varying copper content of bordeaux used in spray on growth of cucumber plants (table 2). (Four pounds of lime in each 50 gallons: *A*, data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

on the growth, however, but rather by a reduction in soluble copper, i. e., a reduction in copper injury. The soluble copper in 4–1–50 bordeaux scorched the foliage so badly as to stunt the plants.

The fact that growth diminished as the lime content of the bordeaux increased above 4–2–50 was not proof that the lime was wholly responsible, however, because both spray load and pH value also increased

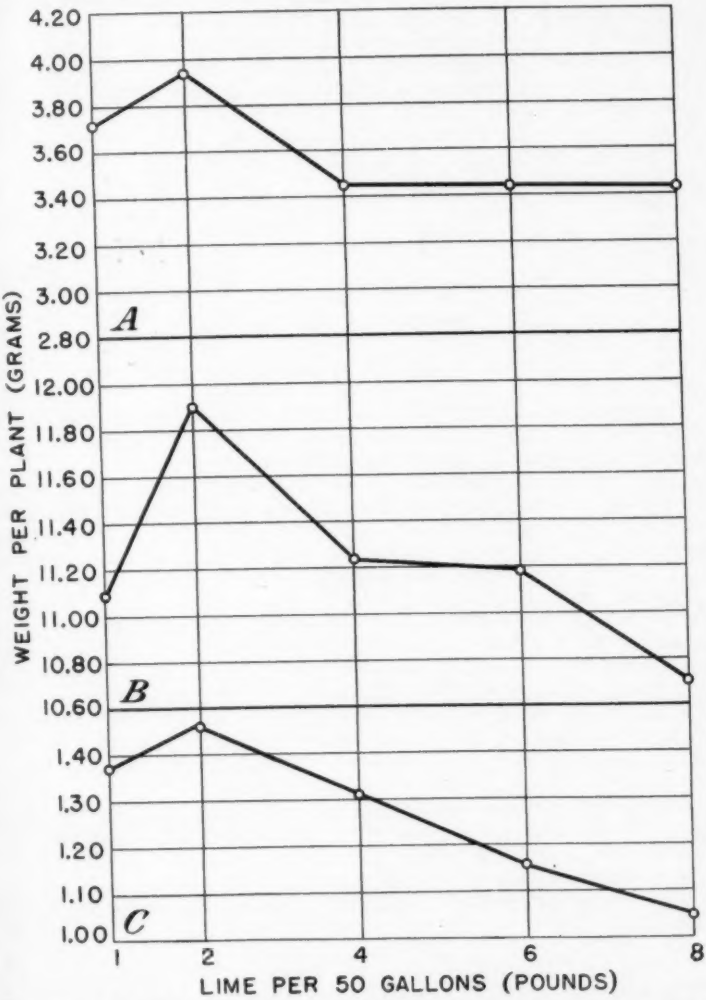

FIGURE 4.—Effect of varying lime content of bordeaux used in spray on growth of cucumber plants (table 2): *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

and both of these are important in the dwarfing caused by the bordeaux as shown later.

If soluble copper compounds do not stunt cucumber foliage seriously or deform the leaves, and if lime does, it can be assumed that the active

agent is the calcium in the lime, or the alkalinity or both. The part played by hydrogen-ion concentration is discussed below.

The effects of excess calcium absorption into the tissues are not clearly understood. Nutritional studies with calcium usually deal with deficiencies. A low-lime soil was put into the greenhouse and fertilized well (at the rate of 2,000 pounds of 5–10–12 per acre). Lime was added to half of this soil at the rate of 3 tons per acre. Three consecutive crops of seedlings were grown in the usual way (table 3).

TABLE 3.—*Effect of liming soil on growth of cucumbers, 1938*

Series No.	Date harvested	Average weight per plant in—	
		Limed soil	Nonlimed soil
		Grams	*Grams*
1	Mar. 18	3.20	5.64
2	Apr. 8	3.50	4.16
3	May 3	7.17	8.18

The plants in the limed soil were drastically dwarfed, and the leaves were deformed in a fashion resembling bordeaux deformation. The dwarfing, as shown by the difference between the plants in the limed and nonlimed soils diminished with succeeding crops. This experiment shows that a high lime content in the soil like a high lime content in a spray will dwarf cucumbers and deform the leaves. It still leaves unanswered, however, the question as to whether the dwarfing is due to excess calcium absorption or to some indirect effect of high alkalinity.

The addition of red cuprous oxide seemed to lessen the dwarfing effect of a lime spray (series 8 and 9, table 1). This would be expected if dwarfing were caused by penetration of calcium ions into the tissues, because the addition of copper oxide to calcium oxide solution would depress the calcium-ion concentration and thus reduce the injury. This reduction in calcium-ion concentration would result from the action of the oxide anion common to both salts. It is possible also that there is a direct antagonism in the tissues between the calcium and copper ions.

Calcium arsenate, which carries calcium but no oxide, served to check the ionization hypothesis. Used alone it reduced growth somewhat (series 6, 6a, 8, and 9, table 1), but, as expected, the addition of red cuprous oxide to it did not reduce the injury appreciably, because copper oxide and calcium arsenate do not carry a common anion.

EFFECT OF WATER

It is difficult to obtain adequate experimental comparisons of materials with and without water, because it is difficult to apply comparable spray loads. Bordeaux and copper-lime dust were compared in the field in 1936 and 1937. Three seedling harvests from six replicate plots were made in 1936 and one harvest from five replicate plots in 1937. The paired average weights in grams per plant for those covered with 25–75 copper-lime dust and bordeaux, respectively,

were as follows: 3.54–3.23, 9.88–7.80, and 24.7–24.2 in 1936; and 12.99–9.88 in 1937. These data show that water aggravates the dwarfing injury from copper sulphate-lime mixtures. Since the water acts as a vehicle, its effect is indirect.

EFFECT OF DILUENTS AND INSECTICIDES

The diluents were used alone and in combination with red cuprous oxide, copper oxychloride, and copper silicate. The materials tried were as follows: Calcium arsenate, lead arsenate, Bancroft clay, Hammil & Gillispie clay, bentonite, Wyogel (Wyoming bentonite), derris, diatomaceous earth (Celite), wheat flour, gypsum, lime, sulphur, and talc (tables 1 and 2).

Of the diluents, wheat flour, talc, diatomaceous earth, and gypsum gave least dwarfing. All of the clays including the bentonites stunted the plants somewhat and deformed the leaves. On this basis it was probably the bentonite diluent in the copper silicate that caused it to stunt the plants. The most injurious clay was Bancroft clay, a material sometimes used as a diluent for fungicides and insecticides (*3*). It is interesting that the addition of red cuprous oxide to a lime spray tended to reduce the injury caused by the lime, but the addition of red cuprous oxide to Bancroft clay spray increased both the stunting and the leafy deformation caused by the clay (table 1, series 4, 4a, 5, 8, 9, and fig. 1). A combination of copper silicate and Bancroft clay was more injurious also than either alone (table 1, series 4, 4a, and fig. 1).

Of the insecticides, derris was much the least injurious, followed by calcium and lead arsenate.

EFFECT OF SPRAY LOAD

A clear picture of the effect of sprays on plants cannot be obtained unless the spray load is held constant. Spray load has been defined (*3*) as the amount of material applied to the leaf. The amount actually retained was not determined in these tests. Since no detergents were added unless otherwise stated, it was assumed that the application and retention of all sprays was essentially proportional to the amount of material in 50 gallons of water.

The data on lime alone indicate that increasing the spray load increased dwarfing (table 1, series 3, 3a, 3b, 8, 9, and table 4).

The three experiments (table 2) varying the copper and lime contents of bordeaux were, among other things, experiments on variable spray load. When curves were plotted for the data (fig. 5) using mixtures of the same hydrogen-ion concentration it became clear that growth generally decreased as spray load increased whether the copper or the lime was varied.

Perhaps the curves in figure 5 would have been more conclusive had they not been plotted from data in which both copper and lime ratios shift. Accordingly a test was made in the greenhouse (table 4) where both spray load and copper-lime ratios were shifted independently. Copper sulphate-lime ratios of 2–1, 1–1, and 1–2 were compared, each with spray loads of 3, 6, and 12 pounds per 50 gallons of water. These data confirm those in figure 5 showing that growth fell off as spray load increased.

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Another test was made in which the spray load was varied by using amendments designed to increase the run-off and thus reduce the spray load. In this test lime at 8–50 was sprayed alone, plus soybean flour, and plus Grasselli spreader-sticker (SS3). The relative run-off for the three mixtures was determined in the laboratory by using a

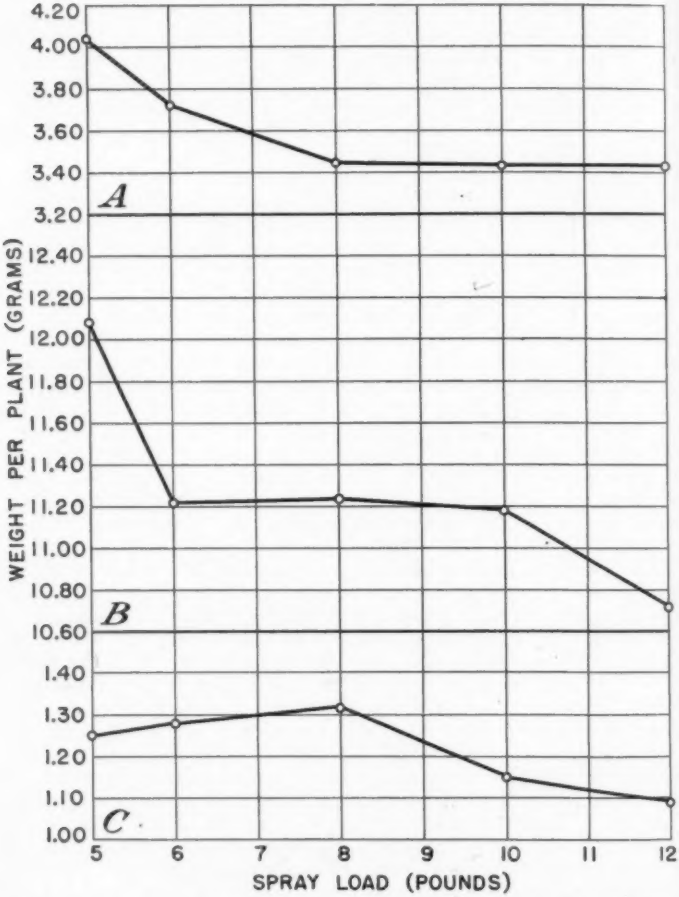

FIGURE 5.—Effect of varying spray load of bordeaux (pounds per 50 gallons) at same pH value on growth of cucumber plants (table 2). *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

cellulose nitrate film on glass and a laboratory sprayer similar to that described by Horsfall, Marsh, and Martin (*5*). The average time in seconds in three trials for the first drop of spray to run down was determined for the three mixtures. These data are compared with those for plant growth of the sprayed plants (table 5).

TABLE 4.—*Effect of spray load and copper lime ratios in bordeaux mixtures on growth of cucumbers*

Copper sulphate-lime ratio	pH	Bordeaux formula [1]	Spray load, quantity per 50 gallons	Average green weight per plant
			Pounds	*Grams*
2:1	11.7	2–1–50	3	3.54
		4–2–50	6	3.40
		8–4–50	12	3.28
Average				3.41
1:1	12.3	1½–1½–50	3	3.49
		3–3–50	6	3.40
		6–6–50	12	2.59
Average				3.16
1:2	12.3	1–2–50	3	3.36
		2–4–50	6	3.03
		4–8–50	12	2.55
Average				2.98
	12.3	0–3–50	3	3.04
		0–6–50	6	3.02
		0–12–50	12	2.92
Average				2.98
Average per spray load			3	3.36
			6	3.21
			12	2.83

[1] Expressed as pounds of copper sulphate and lime per 50 gallons of water.

Both the detergents increased run-off and thus lessened the spray load, which in turn, lessened the dwarfing. This is further evidence that spray load is important in dwarfing.

EFFECT OF HYDROGEN-ION CONCENTRATION

It was interesting to study the effect of hydrogen-ion concentration on growth and leaf injury since lime, which is alkaline, and Bancroft clay, which is acid, both gave stunning and leaf deformation. The effect of hydrogen-ion concentration has been studied (1) by varying the hydrogen-ion concentration of bordeaux, (2) by varying the hydrogen-ion concentration of red cuprous oxide suspensions prepared with different diluents, and (3) by using diluents of different hydrogen-ion concentrations without copper (tables 1 to 4).

TABLE 5.—*Effect of amendments to a lime spray on growth of cucumbers and run-off (i. e., spray load)*

Material	Spray formula [1]	Average green weight per plant	Average time for run-off
		Grams	*Seconds*
Lime alone	8–50	7.08	34.0
Lime+soybean flour	8–1–50	7.58	12.7
Lime+883 [2]	8–0.19–50	7.68	18.7

[1] Expressed as pounds of materials per 50 gallons of water.
[2] Grasselli spreader-sticker.

The hydrogen-ion concentration of the various bordeaux mixtures of approximately equal spray load was plotted against growth of cucumber plants for three experiments (fig. 6). Since no neutral bordeaux was prepared, the growth of the water-sprayed plants was

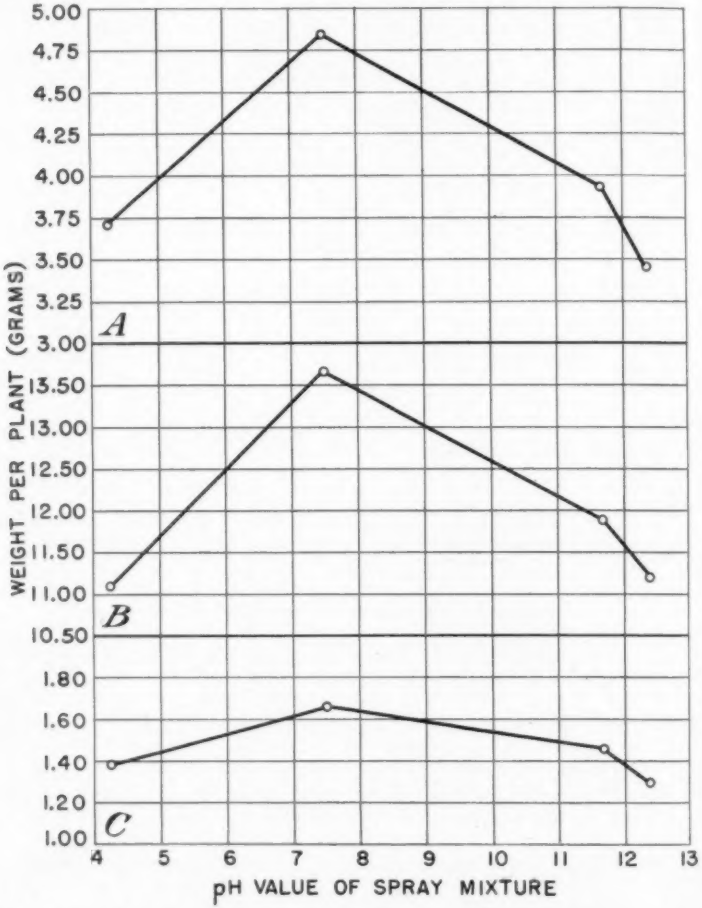

FIGURE 6.—Effect of varying the hydrogen-ion concentration of bordeaux mixture on growth of cucumber plants (table 2). The point for pH 7.5 is located by using the growth of water-sprayed checks: *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

inserted in the curves to establish the approximate shape of the midpart of the curves. Both acid and alkaline bordeaux reduced growth.

The pH value of bordeaux rises sharply from 4.4 to 11.7 as the formula shifts from 4–1–50 to 4–2–50. In other words a single pound

of lime increases the pH value by more than 7 units. Since bordeaux mixture is an unbuffered system, only a small quantity of lime is required to lift the pH value of the mixture practically to that of limewater once the copper has been insolubilized.

This peculiarity of bordeaux left a wide gap in the growth data between pH 4.4 and 11.7—a gap only partially filled by the use of data from water-sprayed plants. The fact that growth fell so rapidly when the pH value increased from 11.7 to 12.3 indicated that the peak of the curve was somewhere below pH 11.7. This was indicated also by the growth of the water-sprayed checks.

A series of sprays over the pH range were prepared using red cuprous oxide as an "insoluble copper" prepared with various diluents (table 2). The spray load was kept constant at 1.2–5–50. The

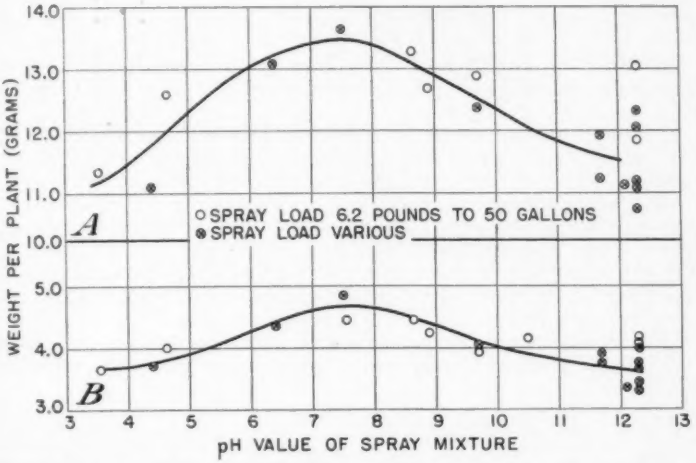

FIGURE 7.—Effect of hydrogen-ion concentration of various spray materials on growth of cucumbers (table 2): *A*, Data from June 1937 experiment; *B*, data from May 1937 experiment.

copper concentration was held constant at 1 pound of metallic copper per 50 gallons.

Curves (fig. 7) were drawn by inspection from three different experiments. The points for red copper oxide with diluents are shown as small circles. For the sake of completeness data from all other materials including bordeaux in the same tests are inserted on the curves as small crosses. The curves are essentially the same as those obtained with bordeaux alone showing poor growth at both high and low pH values. In these curves, however, the gothic shape is rather clearly defined. The optimum growth of cucumber plants was obtained with a spray mixture approximating pH 7.

Other data on the effect of miscellaneous materials (table 1) sprayed by themselves onto cucumber plants are interesting in this connection. As was pointed out above, lime alone, which is alkaline, and Bancroft clay alone, which is acid, both dwarfed cucumbers.Three distinct materials in the same experiment (table 1, series 6 and 6a), acid Bancroft

clay, neutral red cuprous oxide, and slightly alkaline talc, gave the typical curve where growth was smaller with either the acid or alkaline material than with the neutral material. Critical observation of the data shows that the differences were small, but nevertheless the trend was the same in both series as it was in the two larger experiments (table 2) conducted 1 year later. Whatever be the statistical significance of an individual point on a curve, trends are significant especially if they appear in different experiments conducted under different conditions.

It appears to follow then, that growth of cucumbers is influenced by the hydrogen-ion concentration of the spray mixture irrespective of whether copper is present or not. A pH value below neutral probably exaggerates copper injury by aiding copper solubilization. The addition of acid Bancroft clay, for example (series 4, 4a, 5, 8, 9, table 1 and figure 1), to red cuprous oxide or copper silicate stunted cucumber plants and deformed the leaves more than when any of the three materials were used alone. Conversely, a pH value above neutral probably reduces copper injury because it reduces copper solubilization.

The striking relation of hydrogen-ion concentration of the spray mixture to dwarfing explains several observations. The dwarfing effect of the copper silicate was probably an effect of hydrogen-ion concentration because the sample showed a pH value at spray dilution of 8.38. From the curves it is plain that copper silicate at pH 8.38 should cause more dwarfing than red cuprous oxide at pH 6.40. The dwarfing from the various clays used was probably owing to the fact that none of them was near neutral in hydrogen-ion concentration. Some were above neutral, and others were below.

The derris, flour, talc, and gypsum diluents were relatively harmless to the plants because at spray dilution the pH value was practically 7.

DISCUSSION

There is a striking similarity between the factors for dwarfing caused by bordeaux and for the effects of bordeaux on transpiration. Neither factor was very much affected by the insolubilized copper materials. Both were favored by the lime and the water in bordeaux, and both were affected similarly by the acidity or alkalinity of the spray.

The similarities are concerned fundamentally with the effects of the hydrogen-ion concentration of the sprays. It has been suggested that the effect of bordeaux on transpiration (*3*) results from the dissolution of the plant cuticle by acids and alkalis. Increased transpiration is known to dwarf plants. It might be concluded that dwarfing in this case is a simple result of the effect of bordeaux on transpiration. If so, the search for the cause of the dwarfing induced by bordeaux need go no further.

Several facts disturb the simplicity of this conclusion: (1) The seedlings were growing in moist soil in a greenhouse under optimum conditions. Perhaps they could keep themselves supplied with enough water for growth even in the face of a rapid outgo. (2) Red cuprous oxide reduced the dwarfing, but not the transpiration effects of a lime spray. (3) Increasing spray load reduced stomatal tran-

spiration and increased cuticular transpiration, but it uniformly decreased growth.

If dwarfing has other causes than accelerated transpiration, it must be due to an internal absorption of an overdose of copper or calcium from the bordeaux. Horsfall, Magie, and Suit (*4*) report that copper stiffens the tissues of tomatoes. This effect may have caused the small amount of dwarfing occasioned by the insolubilized copper materials. The part played by the copper component of bordeaux was probably small because insolubilized copper compounds did not cause much dwarfing and because copper-free materials did cause it. The lime element in bordeaux cannot be eliminated so readily.

Evidence is presented here and elsewhere (*4*) that calcium penetrates the tissues—hardens, and dwarfs them. All of the materials that showed much dwarfing contained some calcium although it may have been tightly bound up in some of the clays.

Assuming that excess calcium has a dwarfing effect, it is interesting to consider how calcium penetrates the tissue and how it dwarfs growth. The possible avenues of entry are the stomata and the cuticle. If the cuticle is concerned, only those calcium-containing materials with an acid or an alkaline reaction should cause dwarfing, because they are presumably the only ones that would injure the cuticle sufficiently for penetration. Such was the case. Dwarfing was negligible with gypsum and talc, both of which gave an essentially neutral spray. These materials also should have caused dwarfing if the stomata had been the avenue of entry.

Calcium probably reduces growth by curtailing the cell expansion phase. Sayre and Nebel (*8*) have shown that calcium taken in by a pea plant from water culture hardened the tissues of the ovule, presumably by combining with constituents of the middle lamella. It also thickened the cell walls.

The evidence from research on transpiration (*3*) and photosynthesis (*7*) indicates that sprays clog stomata. Therefore, photosynthesis is decreased, and growth is curtailed.

The three basic causes of the dwarfing induced by bordeaux can now be traced outward from known facts as follows: (1) Excessive transpiration of plants is known to cause dwarfing. Therefore, the dwarfing may result from the effect of bordeaux on transpiration, which appears to be a case of water loss through an injured cuticle (*3*). (2) On the other hand, calcium in the lime could pass into the plant through the same injured cuticle. Calcium is known to harden tissues. This could prevent cell enlargement and result in dwarfing. Therefore, the dwarfing in this case may partly accompany and partly be a result of the effect of bordeaux on transpiration. (3) Bordeaux may clog the stomata, curtail photosynthesis, and thus reduce growth.

PRACTICAL ASPECTS

From the standpoint of the practical grower the data presented herein indicate that both acid and alkaline sprays or dusts should be avoided on cucumbers and melons since these plants are dwarfed by them. A neutral bordeaux is an impractical recommendation in the hands of most growers because the margin of safety is too narrow. A

difference of an ounce or two of lime would cause serious copper burn. A small amount of carbonation would give the same result. One of the insolubilized copper compounds would be preferable, although some of these are less satisfactory than others. Calcium arsenate appears to be the best insecticide consistent with expense, although some attention might be paid to making this material so that it has a neutral reaction in water suspension.

Diluents are also important. So-called inert carriers frequently are far from inert when applied to cucurbits. Many of them dwarf the plants. The clays as a group and lime seem to be out of the question from that standpoint. The safest carriers that were tried were diatomaceous earth, gypsum, talc, and wheat flour. Gypsum and diatomaceous earth did not run through the duster as well as talc or wheat flour, but flour is expensive. That leaves only talc.

The precise formula for making dusts or sprays of these materials must await extensive field research.

SUMMARY

There are six symptom types of bordeaux injury on cucurbits: Dwarfing, leaf deformation, yellowing of leaf margins followed by necrosis, scorching of leaf lamina, accelerated transpiration, and defloration.

The copper factor in a bordeaux mixture does not seem to be involved in bordeaux dwarfing unless the copper is solubilized by low hydrogen-ion concentration.

The lime factor definitely dwarfed cucurbits and deformed the leaves.

When water was added to a copper sulphate-lime mixture, dwarfing and leaf deformation were enhanced.

Increasing spray load increased injury.

The most significant factor in dwarfing and leaf deformation appears to be the hydrogen-ion concentration. If the hydrogen-ion concentration of the spray mixture was shifted very far either way from neutral, dwarfing was increased whether copper was present in the spray mixture or not.

The calcium ion also may possibly cause dwarfing by hardening young cells and preventing their enlargement.

The basic cause of dwarfing is discussed. From data available it is suggested that the dwarfing may be due to (1) physiological drought in the tissues induced by the effect of bordeaux transpiration, (2) to action of calcium ions in hardening the tissues, or (3) to reduced photosynthesis, or to a combination of these causes.

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INDIVIDUAL AND BREED VARIATIONS IN PIGS ON RATIONS DEVOID OF VITAMIN D[1]

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INTRODUCTION

That a deficiency of vitamin D often occurs in swine during the winter under farm conditions is suggested from numerous reports received from farmers in Minnesota of so-called fits in pigs. This is supported by the fact that two separate groups of fall pigs raised at the Minnesota Agricultural Experiment Station were later found to be partially or entirely depleted of vitamin D reserves at the time they were placed on experiments which are reported in this paper.

Many experiments have been conducted to determine the improvement in swine rations from additions of calcium, phosphorus, and vitamin D supplements. Unfortunately, it is not possible to interpret the results of the majority of these experiments in terms of the pig's requirement for vitamin D because in some the relationship between these mineral elements and vitamin D was not considered; and in others, the content of these nutrients in the control rations, as well as in the supplements, was not determined. It is of importance in nutrition experiments with swine, as well as in practical pig feeding, to know whether the pig is similar to the rat in not needing vitamin D when the calcium and phosphorus content of the ration is properly adjusted or whether it is similar to the chick in needing vitamin D regardless of the level of these mineral elements.

A series of investigations were conducted on the vitamin D requirement of the pig. It is the purpose of this paper to present data which show that the pig requires vitamin D and that there is considerable variation among breeds and even among pigs of the same breed in this requirement; that the reduced plasma-calcium values, as a result of lack of this vitamin, have a pronounced effect on growth and feed consumption; and that white pigs are less apt to become rachitic than colored pigs under similar conditions in the winter.

REVIEW OF LITERATURE

Bethke, Edgington, and Kick[2] found that there should be at least 0.60 percent of phosphorus in the ration of growing pigs in the absence of added vitamin D. A higher level, however, was not tested. With this percent of phosphorus, growth was satisfactory; and the calcium and inorganic phosphorus levels of the blood serum were not reduced, even though the calcium-phosphorus ratio varied from 1.2 to 3. Greater growth, however, resulted from the addition of cod-liver oil

[1] Received for publication November 7, 1938. Paper No. 1658, Journal Series, Minnesota Agricultural Experiment Station.

[2] BETHKE, R. M., EDGINGTON, B. H., and KICK, C. H. EFFECT OF THE CALCIUM-PHOSPHORUS RELATIONSHIP OF THE RATION ON GROWTH AND BONE FORMATION IN THE PIG. Jour. Agr. Research 47: 331–338, illus. 1934.

to a ration containing 0.45 percent of phosphorus. Apparently this means that the pig requires vitamin D and that a slight deficiency of the vitamin may affect growth without lowering the calcium and inorganic phosphorus levels of the blood. If the pig needs vitamin D, then there must have been storage of the vitamin during the preexperimental period in the pigs receiving the 0.60-percent phosphorus ration to which the vitamin had not been added. In fact, these investigators were of the opinion that the difference they found in ash values of femurs from fall-farrowed pigs as compared with those of spring-farrowed pigs was primarily due to variations in vitamin D storage.

The work of Sheehy [3] furnishes some evidence that the pig stores significant amounts of vitamin D. He concluded that there is considerable storage of the vitamin during the early fall months before the beginning of winter. Although his conclusion is probably correct, his experiments may also demonstrate that the pig requires little vitamin D in the presence of optimum amounts of calcium and phosphorus.

The experiments of Dunlop [4] likewise suggest storage of vitamin D by the pig. A ration containing 0.56 percent of phosphorus with a calcium-phosphorus ratio of 3 was satisfactory for summer but not for winter. In both cases the pigs were kept under confinement from the sun. Although he explained this difference as due to the greater amount of energy required to maintain the animal during winter than summer, it would seem that the difference may have been due to a greater storage of vitamin D by the pigs in the summer test before the start of the experiment than by those in the winter test. From several experiments on the pig's need for vitamin D with various levels of calcium and phosphorus, Dunlop concluded that the pig does not require added vitamin D providing the calcium and phosphorus levels are adequate as, for example, calcium levels of 0.47 or 0.86 percent and a phosphorus level of 0.56 percent. In view of a lack of information of vitamin D storage in his experimental pigs, it appears that such a broad statement as applying to all pigs in general is indeed questionable.

If there is a storage of vitamin D in the body of the pig as a result of solar irradiation or of feeding vitamin D supplements, sufficient to give protection from rickets during the usual experimental period, then the importance of a knowledge of such storage in vitamin D experiments is obvious.

Aubel and Hughes [5] recently reported that a ration containing 0.75 percent of phosphorus and having a calcium-phosphorus ratio of approximately 1 was decidedly unsatisfactory, even more so than a phosphorus level of 0.24 percent and a calcium-phosphorus ratio of 3. The best results were obtained from a ration containing 0.51 percent of phosphorus and a calcium-phosphorus ratio of 1.54. They believed that the results with the ration containing 0.75 percent of phosphorus were owing either to the high level of phosphorus or to the unfavorable calcium-phosphorus ratio. That neither of these explanations

[3] SHEEHY, E. J. COD LIVER OIL IN PIG FEEDING. Jour. Dept. Agr. Irish Free State 32: 202-215. 1933.
[4] DUNLOP, GEORGE. THE CALCIUM, PHOSPHORUS AND VITAMIN D REQUIREMENT OF SWINE. Jour. Agr. Sci. [England] 25: [22]-49, illus. 1935.
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appears correct is indicated by the fact that Dunlop [6] obtained equally good results with a ration containing 0.53 percent of phosphorus and 0.86 percent of calcium as with a ration containing 0.95 percent of phosphorus and 0.79 percent of calcium. If pigs require vitamin D, then in the absence of the vitamin, rations higher in these mineral elements should be beneficial rather than detrimental. The most logical explanation for the performance of the pigs receiving the ration containing 0.75 percent of phosphorus is that the pigs needed vitamin D. This explanation is supported by the data showing low calcium of the blood serum.

It is evident that the literature does not permit a conclusion as to whether the pig needs vitamin D when receiving rations adequate in calcium and phosphorus, and, if there is such a need, whether it is of any practical significance.

EXPERIMENTAL PROCEDURE

FIRST EXPERIMENT

The first experiment, conducted from January 8 to March 12, 1934, involved three lots of five pigs per lot. In each lot there were one Hampshire, two Poland Chinas, and two Duroc-Jerseys. The pre-experimental treatment of the Poland China pigs was not the same as for the other pigs, for the former had been obtained from the South East Minnesota Experiment Station at Waseca, Minn. All lots in this experiment, as well as in the experiments which follow, were housed in a heated swine barn. At no time did any of the pigs have access to sunshine or skyshine for the duration of the tests reported in this paper.

It was desired to use rations which were made up of natural foods to as great an extent as possible and which would give a high level of phosphorus. Corn and tankage were, therefore, used as the basal ingredients. Table 1 shows that a different kind of tankage was used for lot 1 than for lots 2 and 3. Previous tests had shown that tankage (2) had a much greater growth-promoting value than tankage (1). Since the need for vitamin D is dependent to a certain extent upon growth, it was felt that if the pigs required vitamin D, there might be a manifestation of this requirement sooner in lot 2 than in lot 1. The data which follow show that the need for vitamin D was so great that it completely overshadowed any difference in nutritive value of these supplements. The three rations were, therefore, essentially the same, with the exception that the rations for lots 1 and 2 did not contain added vitamin D while the ration for lot 3, used as the control, had an addition of irradiated yeast. Each ration, fed as a mixture, contained approximately 15 percent of protein, 0.65 of phosphorus, and 1.07 of calcium. This gave a calcium-phosphorus ratio of 1.65. Each group received the same quantity of feed.

In addition to the general condition and gross symptoms of the pigs, the level of calcium and inorganic phosphorus in the blood plasma was used to determine the need for vitamin D. The blood for these analyses was obtained from tail bleeding on 2 consecutive days.

[6] DUNLOP, GEORGE. See footnote 4.

TABLE 1.—*Rations used in experiment 1 to determine the vitamin D requirement of the pig*

Ration 1		Ration 2		Ration 3	
Component	Percent	Component	Percent	Component	Percent
Yellow corn	86.00	Yellow corn	86.00	Yellow corn	86.00
Tankage (1)	11.50	Tankage (2)	12.11	Tankage (2)	12.11
Sodium chloride	.50	Sodium chloride	.50	Sodium chloride	.50
Yeast [1]	.10	Yeast [1]	.10	Irradiated yeast [1]	.10
Bonemeal	.96	Limestone	.59	Limestone	.59
Limestone	.16	Tapioca	.70	Tapioca	.70
Tapioca	.78				

[1] The yeast in all rations was increased to 0.15 percent on Feb. 14. The unirradiated yeast used was furnished by Standard Brands, Inc.

RESULTS

At the time the experiment was started, the Poland China pigs were quite unthrifty but were used because other pigs were not available. Two of the pigs in the group from which the six were taken for the experiment had had several tetanuslike convulsions. One of these pigs died during a convulsion, but an examination offered no explanation for its death or for the condition of the others.

However, the results of the blood-calcium analyses, made 1 week after the start of the experiment, readily explained their behavior. Table 2, in which all of the calcium and inorganic phosphorus determinations are given, shows that the Poland China pigs, Nos. 1, 2, 6, and 7, in lots 1 and 2 were suffering from slight to very great diminutions of blood calcium. The plasma calcium of one other pig in each of these lots, Nos. 5 and 9, respectively, was also low. The convulsions in the two pigs were undoubtedly a manifestation of low calcium tetany. The vitamin D stores in the pigs evidently had been depleted during the early part of the winter. Therefore, low-calcium tetany can be produced in pigs with a ration adequate in calcium but lacking in vitamin D or, as has since been reported by Hughes,[7] with a ration adequate in vitamin D but low in calcium. It is considered that the plasma calcium levels of the Poland China pigs, Nos. 11 and 12, in lot 3 were probably also low before the start of the experiment and that the vitamin D in the irradiated yeast had returned these values essential to normal. This statement is made in view of the similar initial unthrifty appearance of the Poland China pigs in all three lots.

During the course of the experiment, two other pigs, Nos. 4 and 7, were observed to have the tetanuslike convulsions. No. 7 had such severe convulsions that it was removed from the experiment on February 26. Four weeks after the start of the experiment pig No. 8 was dropped from the experiment because of a skin disease. Since it had been planned to feed all lots the same amounts of feed, the corresponding litter mates in the other lots were also dropped.

The data in table 2 show that, as the experiment progressed, the blood-plasma calcium of all of the pigs, with the exception of those in lot 3, decreased to very low levels. The change in the inorganic phosphorus, however, was relatively slight and highly variable. Four

[7] HUGHES, E. H. THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF THE BLOOD SERUM OF SWINE. Jour. Agr. Research 53: 267-279. 1936.

weeks after the first determinations, the plasma calcium of the pigs in lot 3 also decreased. Accordingly on February 14, the irradiated yeast was increased to 0.15 percent. That this was beneficial is evident from the higher plasma calcium values of lot 3 on February 27 and 28. A similar increase of the unirradiated yeast in the ration of lots 1 and 2 on February 14 was without benefit.

TABLE 2.—*Calcium and inorganic phosphorus in 100 cc of blood plasma of pigs at different times during experiment 1 to determine their vitamin D requirement*

Lot No.	Pig No.	Jan. 14–15		Feb. 11–12		Feb. 27–28 [1]		Mar. 11–12	
		Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	1	9.48	7.43	5.66	6.76	5.59	7.09	10.78	7.60
	2	6.94	7.49	5.97	7.02	5.23	7.41	--------	--------
	3	10.78	7.81	--------	--------	--------	--------	--------	--------
	4	10.57	8.55	8.85	7.87	5.59	7.94	11.08	7.81
	5	9.74	7.14	7.82	5.78	6.47	5.62	11.08	8.06
Mean		9.50	7.68	7.08	6.86	5.72	7.02	10.98	7.82
2	6	6.84	7.52	4.43	7.22	6.36	6.85	[2] 10.47	--------
	7	5.39	6.69	5.35	5.85	5.13	9.78	--------	--------
	8	10.16	7.52	--------	--------	--------	--------	--------	--------
	9	9.90	7.14	8.65	7.46	5.85	7.58	11.08	8.06
	10	10.16	7.07	9.37	6.60	7.08	8.55	10.93	7.43
Mean		8.49	7.19	6.95	6.78	6.11	8.19	[3] 11.01	7.75
3	11	10.68	7.63	9.57	7.14	9.75	8.37	9.70	7.04
	12	10.16	7.41	9.78	7.41	10.67	9.71	--------	--------
	13	11.30	6.92	--------	--------	--------	--------	--------	--------
	14	11.19	7.81	9.68	7.17	9.85	8.03	11.03	8.03
	15	10.57	6.54	10.09	7.14	10.67	8.30	10.88	7.81
Mean		10.78	7.26	9.78	7.22	10.24	8.60	10.54	7.63

[1] Nos. 2, 7, and 12 were bled on Feb. 26.
[2] No. 6 was bled on Mar. 4.
[3] Mean for Nos. 9 and 10.

By February 28 all the pigs in lots 1 and 2 had severe cases of rickets, as judged from their low blood calcium. This was accompanied by harsh hair coats and an unthrifty appearance. On this date the unirradiated yeast in their ration was replaced by irradiated yeast. This had an immediate effect in raising the blood calcium, as seen in the normal value for pig No. 6 4 days after the change in yeast. The normal values for the remaining pigs 2 weeks after the change in the ration confirmed the result. During this period there was also an improvement in appetite.

The pigs in lots 1 and 2 had poor appetites and, consequently, made subnormal growth. During the 9 weeks of the experiment the pigs ate an average of only 2.69 pounds of feed daily. The feed consumption and growth records are given in table 3. The slow growth of all of the pigs in lot 3, except No. 14, can be explained by the fact that they were limited to the same feed intake as eaten by each of the other lots. It is felt that No. 14, being larger than the other pigs in the lot, was able to obtain more than its share of the feed.

TABLE 3.—*Growth and feed data of pigs at different times during experiment 1 to determine their vitamin D requirement*

Date	Weight of pigs in lot 1					Weight of pigs in lot 2					Weight of pigs in lot 3					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	Average daily feed consumed per pig [1]
	Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
Jan. 8	39	42	62	47	57	40	40	59	47	57	33	35	68	56	51	.....
Jan. 15	42	46	73	55	62	42	41	65	53	68	37	38	74	64	58	2.86
Jan. 22	45	47	77	60	63	45	39	67	56	73	36	40	79	72	61	2.17
Jan. 29	44	47	83	66	63	45	41	65	60	81	39	45	81	81	62	2.46
Feb. 5	44	51	95	75	72	48	40	67	70	90	40	50	86	91	67	2.31
Feb. 12	47	52	.....	82	73	44	42	.....	79	98	41	54	.....	97	74	2.68
Feb. 19	48	57	.....	96	77	47	45	.....	87	107	46	60	.....	109	81	2.54
Feb. 26	53	61	.....	107	78	50	42	.....	93	117	44	68	.....	119	90	2.86
Mar. 5	51	.....	.....	107	85	51	.....	.....	95	120	50	.....	.....	130	101	3.33
Mar. 12	57	.....	.....	116	93	.....	.....	.....	108	136	48	.....	.....	145	112	3.38

[1] All lots were fed equal amounts.

SECOND EXPERIMENT

During the course of the first experiment the work of Dunlop [8] came to the authors' attention. He found that the pigs in his experiments required no vitamin D from weaning to bacon weight when receiving rations containing 0.56 percent of phosphorus and having calcium-phosphorus ratios of 0.83 to 1.54. The only explanation which appeared plausible for his finding as contrasted with that of the present writers was that the pigs in his experiments had stored sufficient vitamin D to protect them from rickets during the experimental period. This was suggested by the fact that the pigs used by Dunlop were of the Large White breed whereas in the writers' experiment only colored pigs were used. It was felt that there might be a greater storage of vitamin D by nonpigmented pigs than by those which are pigmented, and also that there might be an hereditary difference among breeds in their requirement for this vitamin.

Accordingly, two lots of pigs were placed on experiment December 12, 1935. Lot 1 contained one Duroc-Jersey, one Poland China, five Hampshires, and five Chester Whites. Lot 2, which received the control vitamin D-containing ration, consisted of two Poland Chinas and four Duroc-Jerseys. The initial weight of the Chester Whites was 50 pounds; of the Duroc-Jerseys and Poland Chinas, 55 pounds; and of the Hampshires, 75 pounds. They were individually fed twice daily.

The ration fed lot 1 consisted of yellow corn, 86.84 percent; tankage, 11.80 percent; sodium chloride, 0.50 percent; bonemeal, 0.26; limestone, 0.45 percent; and unirradiated yeast, 0.15 percent. The same ration was fed to lot 2 except irradiated yeast was used in place of the unirradiated yeast. The percentages of protein, calcium, and phosphorus in these rations were the same as those used in the first experiment.

[8] DUNLOP, GEORGE. See footnote 4.

Since the first experiment showed that a depletion of vitamin D resulted in a marked change only in the calcium content of the blood plasma, only calcium analyses of the blood plasma from single blood samples were made in experiment 2.

Results

The plasma-calcium analyses are shown in table 4. The reduction in plasma-calcium values, as a result of a lack of vitamin D, had other pronounced effects on the animals. The effects on growth and feed consumption, are presented for each pig for biweekly periods in tables 5 and 6.

TABLE 4.—*Calcium in 100 cc of blood plasma of pigs at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No.	Pig No.	Breed	Dec. 11	Jan. 9	Mar. 3	Mar. 31
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
1	1	Duroc-Jersey	9.26	10.14	6.91	
	2	Poland China	8.97	10.67	5.23	
	3	Hampshire	9.74	10.29	7.87	
	4	do	9.89	10.86	6.87	
	5	do	10.23	10.67	5.96	
	6	do	10.28	9.85	6.82	
	7	do	10.28	10.77	10.05	10.18
	8	Chester White	10.23	10.96	10.28	10.49
	9	do	9.79	10.86	10.65	10.23
	10	do	9.94	10.58	10.51	10.07
	11	do	9.79	10.58	9.37	8.22
	12	do	9.74	10.19	10.05	9.86
2	13	Poland China	9.31	10.09		
	14	do	9.70	10.82		
	15	Duroc-Jersey	9.21	9.90		
	16	do	9.26	10.29		
	17	do	9.01	10.43		
	18	do	9.89	10.62		

TABLE 5.—*Average daily gain in weight per pig at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No.	Pig No.[1]	Dec. 12–26	Dec. 26–Jan. 9	Jan. 9–23	Jan. 23–Feb. 6	Feb. 6–20	Feb. 20–Mar. 6	Mar. 6–20	Mar. 20–Apr. 3
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	1.21	1.14	1.64	1.93	1.79	0.86		
	2	1.49	.93	1.36	1.49	1.21	[2] −.14		
	3	1.57	.86	1.07	1.71	1.29	[3] −.79		
	4	1.29	.93	1.79	1.71	1.93	.71		
	5	1.93	1.43	1.49	1.79	1.07	.86		
	6	2.14	1.07	.43	1.43	1.71	.43		
	7	2.07	1.21	1.93	1.49	1.29	1.49	2.57	1.86
	8	1.29	1.29	1.36	1.93	2.00	1.79	1.79	2.43
	9	1.79	1.14	1.93	1.79	2.14	1.71	1.49	1.93
	10	1.57	.86	1.71	1.93	1.36	1.57	1.64	1.57
	11	1.57	1.43	1.86	.64	2.21	1.43	.71	1.71
	12	1.29	.86	.93	.21	1.43	1.21	1.43	1.79
2	13	1.14	1.07	1.00	1.21	1.14	1.86	1.93	
	14	1.00	1.21	1.29	1.49	1.57	1.64	1.43	
	15	1.21	1.00	1.43	1.71	1.79	1.93	1.86	
	16	1.07	1.43	1.79	1.64	2.07	2.07	2.00	
	17	1.57	1.49	1.79	2.00	1.86	2.00	2.07	
	18	1.00	.79	1.43	1.07	1.21	1.57	2.07	

[1] See table 4 for breeds of the pigs.
[2] Represents average daily loss of weight.

TABLE 6.—*Average daily feed consumption per pig at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No.	Pig No.[1]	Dec. 12-26	Dec. 26-Jan. 9	Jan. 9-23	Jan. 23-Feb. 6	Feb. 6-20	Feb. 20-Mar. 6	Mar. 6-20	Mar. 20-Apr. 3
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	2.48	3.32	4.41	5.28	6.04	5.66	4.80	4.80
	2	2.81	3.29	3.75	4.26	3.85	2.39	2.21	4.16
	3	3.69	3.73	4.15	5.48	5.37	1.75	4.46	.....
	4	4.69	4.76	5.51	5.64	6.52	5.75	2.73	.....
	5	3.74	4.65	5.16	5.83	5.14	4.37	4.09	4.70
	6	4.31	4.05	4.18	5.09	5.39	4.73	4.36	5.08
	7	4.46	5.46	6.34	6.53	7.93	7.50	8.49	8.64
	8	3.00	3.74	4.09	5.13	6.19	6.51	7.19	8.65
	9	3.16	4.05	5.23	5.77	6.38	6.99	7.55	8.04
	10	3.02	3.50	4.44	5.21	5.34	6.06	6.57	7.70
	11	3.14	4.16	5.03	5.76	6.61	6.69	4.49	7.45
	12	2.51	3.09	3.08	3.39	4.26	4.65	5.82	6.27
2	13	2.09	3.14	3.23	3.51	3.86	4.84	6.34	.....
	14	2.41	3.46	4.00	4.11	4.96	5.22	6.44	.....
	15	2.92	3.51	4.78	5.06	5.91	7.18	8.11	.....
	16	2.79	4.34	5.19	5.22	5.57	7.18	8.07	.....
	17	2.79	4.34	5.19	5.22	6.57	6.62	7.29	.....
	18	1.85	2.73	3.75	4.25	5.34	5.59	7.34	.....

[1] See table 4 for breeds of the pigs.

The fact that 14 of the 18 pigs showed less than 10 mg of calcium per 100 cc of blood plasma on December 11, before the experiment was started, suggested that these pigs were becoming rachitic. However, 4 weeks later, when the next determinations were made, there had been a significant increase in the calcium content of the blood plasma of all of these pigs. Therefore, it seems probable that the low plasma-calcium levels on the earlier date were the result of rations inadequate in calcium. As the experiment progressed, it was apparent that several of the pigs in lot 1, Nos. 2, 3, 4, 5 and 6, were becoming rachitic. This was indicated by their lameness, loss of appetite, and slow growth or even loss in weight. Pigs Nos. 2, 3, and 4 also had several convulsions. Plasma-calcium determinations on some of these pigs revealed that the calcium of the blood was gradually decreasing. Therefore, on March 3, calcium analyses were made of the blood plasma of all the pigs in lot 1. Similar analyses were not made for lot 2 because they were receiving 0.15 percent of irradiated yeast, which, in the first experiment, not only protected the pigs from rickets but also cured severe rickets as well. The results of these analyses indicate that the Duroc-Jersey, Poland China, and the Hampshires, Nos. 3 to 6, inclusive, had very low plasma-calcium values. The records of feed intake and growth show the effects of their rachitic condition. In their behavior and physical appearance they resembled the rachitic pigs in the first experiment. With the exception of Chester White pig No. 11, all the pigs of this breed and Hampshire pig No. 7 had normal values for plasma calcium. When calcium analyses of the blood plasma were again made on March 31, 110 days after the start of the experiment, only Chester White pigs No. 12 and No. 11 showed a reduction in

plasma calcium. However, growth and feed intake of these two pigs were satisfactory for the period from March 20 to April 3.

The experiment was terminated on April 3 because facilities were no longer available for continuing it. Had the pigs which were not at this time rachitic been continued on the diet for a longer period, it is believed that they would have eventually become so. That it was probably a question of greater storage of vitamin D by the white pigs as compared with those which were pigmented is supported by the fact that pig No. 7, the only Hampshire which did not become rachitic, was almost entirely white.

THIRD EXPERIMENT

In view of the results of the second experiment, it was desired to investigate further the difference in the vitamin D requirement of white and colored pigs. For this purpose two lots of six pigs each, each pig averaging approximately 45 pounds in weight, were started on experiment December 21, 1936. Lot 1 was made up of six Chester White pigs and lot 2 of three Duroc-Jerseys and three Poland Chinas. The previous treatment of all the pigs had been the same. Neither lot was allowed access to sunlight. The ration self-fed to both lots consisted of corn, 86 percent; tankage, 12 percent; salt, 0.50 percent; bonemeal, 1.0 percent; and limestone, 0.50 percent. The calcium, phosphorus, and protein in the ration were 1.05, 0.63, and 16.60 percent, respectively. Individual weights of the pigs and the feed eaten by each group were recorded at 2-week intervals. As in the second experiment, the calcium content of the blood plasma of the pigs was used as the index of the need for vitamin D. The calcium, growth, and feed data are shown in tables 7-8, and 9, respectively.

TABLE 7.—*Calcium in 100 cc of blood plasma of pigs at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No.	Pig No.	Breed	Dec. 21	Jan. 18	Feb. 2	Feb. 8	Feb. 15	Mar. 2
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	1	Chester White	11.64	12.36			9.72	9.77
	2	do	10.58	11.02			9.67	9.11
	3	do	10.53	11.56			10.59	9.72
	4	do	11.59	11.61			10.13	9.06
	5	do	11.27	11.34			9.57	8.24
	6	do	10.95	11.08			9.46	9.16
Mean			11.09	11.50			9.86	9.18
2	7	Duroc-Jersey	10.05	9.24	8.59	8.23	6.96	
	8	do	10.95	9.26	7.76	7.24	6.65	
	9	do	10.69	10.27	8.23	7.40	6.92	
	10	Poland China	11.64	10.64	9.31	8.90	7.42	
	11	do	11.01	9.63	8.12	7.81	7.37	
	12	do	11.33	9.84	7.76	6.42	6.04	
Mean			10.95	9.81	8.30	7.67	6.89	

TABLE 8.—*Average daily gain in weight per pig at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No.	Pig No.[1]	Dec. 21–Jan. 4	Jan. 4–18	Jan. 18–Feb. 1	Feb. 1–15	Feb. 15–Mar. 1
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	1.00	1.07	0.79	1.14	1.36
	1	1.00	1.00	1.21	1.36	1.50
	3	1.29	1.21	1.43	1.57	.71
	4	1.43	1.07	1.14	1.14	1.43
	5	1.50	1.21	1.50	1.79	1.93
	6	.93	.64	1.14	.86	1.00
Mean		1.19	1.03	1.20	1.31	1.32
2	7	.29	.36	.29	.64	
	8	.79	.29	.50	.79	
	9	1.29	1.21	1.00	.86	
	10	1.14	1.43	1.07	1.50	
	11	1.21	.93	1.00	.57	
	12	.79	.71	.43	.29	
Mean		.92	.82	.72	.78	

[1] See table 7 for breeds of the pigs.

TABLE 9.—*Average daily feed consumed per pig at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No.	Dec. 21–Jan. 4	Jan. 4–Jan. 18	Jan. 18–Feb. 1	Feb. 1–Feb. 15	Feb. 15–Mar. 1
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	2.68	3.16	3.56	4.17	4.45
2	2.61	3.05	3.24	3.23	

RESULTS

The data in table 7 show that 28 days after the start of the third experiment the blood-calcium value for each of the Chester White pigs was slightly higher than when the pigs were put on the experiment. After the test had been in progress for 56 days, there was a reduction from the former average value of 11.5 mg to 9.86 mg; 2 weeks later there was a further reduction to 9.18 mg. At this time all of the pigs were quite sore-footed. Some were buckling over on the front pasterns and were becoming bowlegged, both in front and behind.

Table 7 reveals quite a different picture for the Duroc-Jersey and Poland China pigs in lot 2. This table shows that it required twice as long for the blood calcium of the Chester White pigs to decrease to essentially the same level as that of the colored pigs. Two of the possible explanations of this result are as follows: Either there was a greater storage of vitamin D in the Chester White pigs or their requirement for the vitamin was less. If it can be assumed that similar reduced levels of blood calcium in the two groups of pigs is indicative of similar residual stores of vitamin D, then the requirement of the Chester White pigs for the vitamin was actually less, as evidenced by the slower rate of decrease of their blood calcium. Some variability also existed in this respect among pigs of the same breed in lot 2. In another study of the vitamin D requirement of growing pigs, two Duroc-Jersey pigs, litter mates, did not become rachitic until 97 days after the start of the experiment. Ten other pigs, which had been under a similar preexperimental environment, became rachitic in 28

days. This suggests that there may be important hereditary differences in the vitamin D requirement of pigs, as Gowen[9] found to be the case with rats.

There was a gradual reduction in blood calcium of the Duroc-Jersey and Poland China pigs to 6.89 mg. Some of these pigs were lame, but this condition was not characteristic of the entire group. It was clearly evident from the blood picture and from the records on growth and feed intake, tables 8 and 9, that these pigs were rachitic and were reaching a level of blood calcium where convulsions could be expected. Both lots were, therefore, exposed to the sun, the exposure bringing about complete recovery.

The results of the third experiment confirm the results of the second experiment showing that white pigs are much less apt to become rachitic under confinement during the winter-feeding period than colored pigs. The explanation for the fact that the white pigs in the third experiment became rachitic in 56 days while most of the white pigs in the second experiment had not become rachitic even after 110 days is that the pigs in the earlier experiment had probably received more vitamin D either through solar irradiation or their feed before the start of the test. This is further supported by the fact that it likewise required longer for the colored pigs in the second test to become rachitic than in the third test.

SUMMARY AND CONCLUSIONS

The growing pig's requirement for vitamin D was investigated during the winters of 1935, 1936, and 1937 with fall-farrowed pigs of the Chester White, Duroc-Jersey, Hampshire, and Poland China breeds of swine. The basal ration used in the experiments contained approximately 0.64 percent of phosphorus and a calcium-phosphorus ratio of approximately 1.65.

The results of these tests showed conclusively that the pigs required added vitamin D after the body stores of the vitamin had been depleted. With the exception of two Duroc-Jersey pigs, all of the colored pigs, having access to the outside during the fall before being placed under confinement, stored sufficient vitamin D to protect them from rickets for periods of only 4 to 8 weeks. White pigs, of the Chester White breed and Hampshire pig No. 7, which was nearly white, raised under the same environment as the colored pigs, were protected for approximately twice as long.

A depletion of vitamin D resulted in a reduction of the blood-plasma calcium from between 10 and 12 mg. per 100 cc. of blood plasma to below 6 mg., the extent of reduction depending on the length of time the pigs were kept on the vitamin D-free ration. When low blood-calcium values were reached, calcium-deficiency tetany was manifested. The reductions in blood calcium were accompanied by a gradual loss of appetite and an unthrifty appearance, characterized by emaciation, a rough hair coat and, usually but not always, lameness. An almost complete loss of appetite invariably resulted with the onset of low-calcium tetany.

[9] GOWEN, JOHN. INHERITANCE AS IT AFFECTS SURVIVAL OF RATS FED A DIET DEFICIENT IN VITAMIN D. Genetics 21: 1–23, illus. 1936.

BACTERICIDAL EFFICIENCY OF CERTAIN ANILINE DYES[1]

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INTRODUCTION

During the last few years the writer has had occasion to make a considerable number of tests of the bactericidal efficiency of various aniline dyes. The ultimate object was to ascertain the utility of these dyes in the preparation and preservation of biological products to be used for the prophylaxis or diagnosis of animal diseases. The biological products particularly involved were hog cholera virus, hog cholera vaccine, and stained antigens for the detection of pullorum disease in fowls and Bang's disease in cattle. Accordingly, use was made of *Escherichia coli*, *Salmonella pullorum*, and *S. suipestifer* as test organisms in addition to *Staphylococcus aureus* and *Eberthella typhi*, which are more commonly used in testing disinfectants. With the exception of *Staphylococcus aureus*, all the test organisms were Gram-negative. The present paper reports the results of representative bactericidal tests of readily available dyes, made in order to select those most suitable for experiments with the individual biological products.

EXPERIMENTS WITH AQUEOUS SOLUTIONS

The initial experiments were conducted on solutions of dyes to which no extraneous organic matter had been added other than that introduced with the cultures of micro-organisms. Bactericidal efficiency was determined by the Rideal-Walker technique, modified as follows: Beef-infusion broth adjusted to pH 7.2 was used instead of R–W standard broth; a 1-cc pipette graduated into tenths was employed for measuring the 0.5 cc of culture required for the test; the temperature at which the experiments were conducted was 25° C. instead of 15° to 18°; dilutions were made in sterile Erlenmeyer flasks instead of in cylinders; fixed quantities of the stock solution of disinfectant were added to varying quantities of water, both being measured by sterile standardized pipettes; several test organisms were used in addition to *Eberthella typhi* (*Bacillus typhosus*); the total time of exposure was increased from 15 minutes to 60 minutes; finally, no phenol coefficients were calculated, bactericidal efficiency being measured by the concentration of disinfectant required to kill the test organism. No attempt was made to neutralize dye carried over into subcultures, but whenever it seemed necessary secondary subcultures were made from the original subcultures.

In table 1 are shown representative results obtained with various commercial dyes in aqueous solution. In those instances in which the dye content was known it is stated, but the concentrations given in the table are based on total weight of the commercial dye without regard to the percentage of actual dye therein.

[1] Received for publication November 25, 1938.

TABLE 1.—*Bactericidal efficiencies of various commercial aniline dyes in aqueous solution*[1]

Dye	Dilution of dye[2]	Results after exposure for[3]—						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
Auramine 0	1-200	−	−	−	−	−	−	*Eberthella typhi.*
	1-400	+	+	+	−	−	−	
	1-600	+	+	+	+	+	+	
	1-400	+	−	−	−	−	−	*Salmonella suipestifer.*
	1-600	+	+	−	−	−	−	
	1-800	+	+	+	−	−	−	
	1-1,000	+	+	+	+	−	−	
	Saturated solution.	+	+	+	+	+	+	*Staphylococcus aureus.*
Basic fuchsin (92 percent dye).	1-400	+	+	+	−	−	−	*Escherichia coli.*
	1-600	+	+	+	+	−	−	
	1-800	+	+	+	+	+	+	
	1-400	+	+	−	−	−	−	*Salmonella suipestifer.*
	1-600	+	+	+	−	−	−	
	1-800	+	+	+	+	−	−	
	1-1,000	+	+	+	+	+	+	
	1-400	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-600	+	+	+	+	−	−	
Malachite green (76-percent dye).	1-1,200	−	−	−	−	−	−	*Eberthella typhi.*
	1-1,600	+	+	−	−	−	−	
	1-2,000	+	+	+	+	+	−	
	1-800	+	+	−	−	−	−	*Escherichia coli.*
	1-1,000	+	+	+	+	+	+	
	1-1,200	+	−	−	−	−	−	*Salmonella suipestifer.*
	1-1,600	+	+	−	−	−	−	
	1-2,000	+	+	+	+	+	+	
	1-4,000	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-8,000	+	+	+	+	+	+	
Brilliant green (94-percent dye).	1-1,200	+	+	−	−	−	−	*Escherichia coli.*
	1-1,400	+	+	+	+	+	+	
	1-1,200	−	−	−	−	−	−	*Salmonella suipestifer.*
	1-1,400	+	+	−	−	−	−	
	1-1,600	+	+	+	+	+	−	
	1-20,000	+	+	−	−	−	−	*Staphylococcus aureus.*
	1-24,000	+	+	+	+	+	−	
Hofmann's violet	1-200	+	+	+	+	+	+	*Escherichia coli.*
	1-400	+	+	−	−	−	−	
	1-800	+	+	+	−	−	−	*Salmonella suipestifer.*
	1-1,200	+	+	+	+	+	−	
	1-2,000	+	+	+	−	−	−	*Staphylococcus aureus*
	1-4,000	+	+	+	+	+	+	
Methyl violet 2B (81-percent dye).	1-2,000	+	+	+	+	−	−	*Eberthella typhi.*
	1-3,000	+	+	+	+	+	+	
	1-200	+	+	+	−	−	−	*Escherichia coli.*
	1-400	+	+	+	+	+	−	
	1-1,200	+	+	−	−	−	−	*Salmonella suipestifer.*
	1-1,600	+	+	+	+	+	+	
	1-2,000	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-3,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	+	+	
Crystal violet (88-percent dye).	1-1,000	+	+	−	−	−	−	*Eberthella typhi.*
	1-2,000	+	+	+	+	+	+	
	1-100	+	+	+	−	−	−	*Escherichia coli.*
	1-200	+	+	+	+	+	+	
	1-500	+	+	+	+	+	−	*Salmonella pullorum.*
	1-750	+	+	+	+	+	+	
	1-500	+	+	+	+	+	−	*Salmonella suipestifer.*
	1-750	+	+	+	+	+	+	
	1-3,000	+	+	−	−	−	−	*Staphylococcus aureus.*
	1-4,000	+	+	+	+	+	+	
Victoria blue	1-200	+	+	+	−	−	−	*Salmonella suipestifer.*
	1-400	+	+	+	+	+	+	
	1-4,000	+	−	−	−	−	−	*Staphylococcus aureus.*
	1-6,000	+	+	+	+	−	−	

[1] Experiments conducted at 25° C.
[2] On basis of commercial dye.
[3] Growth, (+); no growth, (−).

All the dyes shown in table 1 are basic dyes and, with the exception of auramine 0, a diphenylmethane compound, all are triphenylmethane derivatives. Basic fuchsin, a simple triamino derivative, showed comparatively low bactericidal activity, which was approximately

the same against Gram-positive and Gram-negative organisms. All the other dyes carry varying heavy substitutions in the amino groups. Except auramine 0, all manifested much greater bactericidal activity than basic fuchsin and much greater activity against Gram-positive than against Gram-negative organisms. Auramine 0 is noteworthy as an exception to the usually accepted rule that basic dyes are more effective against Gram-positive than against Gram-negative organisms.

Additional data, not presented in table 1 but obtained in the same manner, are as follows: Ethylated crystal violet (ethyl green) was less than one-tenth as efficient as crystal violet itself, and ethyl violet, a homologue of crystal violet, was entirely ineffective in saturated solution; sulphonated brilliant green (light green SF yellowish) was ineffective in 20-percent solution; the thiazine dyes thionine and methylene blue were ineffective, the former in saturated solution and the latter in 2-percent solution; the disazo dye Bismarck brown was ineffective in 1-percent solution.

In table 2 are shown results of experiments in which phenol or orthocresol was added to solutions of some of the same dyes. As in table 1, dye concentration is based on total weight of commercial dye.

TABLE 2.—*Influence of phenol or orthocresol on bactericidal efficiencies of dyes* [1]

Dye	Dilution of dye [2]	Results after exposure for [3]—						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
Basic fuchsin used with Phenol (1–1,000).	1–400	+	+	−	−	−	−	*Escherichia coli.*
	1–600	+	+	+	−	−	−	
	1–800	+	+	+	+	+	+	*Salmonella suipestifer.*
	1–700	+	+	−	−	−	−	
	1–800	+	+	+	−	−	−	
	1–900	+	+	+	+	−	−	
Malachite green used with—								
Phenol (1–1,000)	1–1,200	+	+	+	−	−	−	*Escherichia coli.*
	1–1,400	+	+	+	+	+	+	
Orthocresol (1–1,000)	1–2,000	+	+	+	−	−	−	
	1–2,400	+	+	+	+	+	+	
Brilliant green used with—								
Phenol (1–1,000)	1–1,200	−	−	−	−	−	−	*Escherichia coli.*
	1–1,600	+	+	+	+	+	+	
Orthocresol (1–1,000)	1–1,600	+	+	−	−	−	−	*Salmonella suipestifer.*
	1–2,000	+	+	+	+	+	+	
Phenol (1–1,000)	1–1,400	+	−	−	−	−	−	
	1–1,600	+	+	+	+	+	+	
	1–16,000	+	+	+	+	−	−	*Staphylococcus aureus.*
	1–20,000	+	+	+	+	+	−	
	1–24,000	+	+	+	+	+	+	
Orthocresol (1–1,000)	1–18,000	+	+	−	−	−	−	
	1–24,000	+	+	+	+	+	+	
Methyl violet 2B used with—								
Phenol (1–1,000)	1–2,000	+	−	−	−	−	−	*Eberthella typhi.*
	1–4,000	+	+	+	+	+	+	
Orthocresol (1–1,000)	1–4,000	−	−	−	−	-	−	
	1–6,000	+	+	+	+	+	+	
Phenol (1–1,000)	1–800	+	−	−	−	−	−	*Escherichia coli.*
	1–1,600	+	+	+	+	−	−	
	1–2,400	+	+	+	+	+	+	
Orthocresol (1–1,000)	1–3,000	+	−	−	−	−	−	
	1–4,000	+	+	+	−	−	−	
	1–5,000	+	+	+	+	+	−	
Phenol (1–1,000)	1–2,000	+	+	−	−	−	−	*Salmonella suipestifer.*
	1–3,000	+	+	+	−	−	−	
	1–4,000	+	+	+	+	−	−	
	1–5,000	+	+	+	+	+	−	
Orthocresol (1–1,000)	1–4,000	+	+	−	−	−	−	
	1–5,000	+	+	+	−	−	−	
Phenol (1–1,000)	1–3,000	+	−	−	−	−	−	*Staphylococcus aureus.*
	1–4,000	+	+	+	+	+	+	

See footnotes at end of table.

TABLE 2.—*Influence of phenol or orthocresol on bactericidal efficiencies of dyes*—Continued

Dye	Dilution of dye	Results after exposure for—						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
Crystal violet used with—								
Phenol (1-1,000)	1-2,000	+	−	−	−	−	−	*Eberthella typhi.*
	1-4,000	+	+	−	−	−	−	
	1-6,000	+	+	+	+	+	+	
Phenol (equal weight)	1-2,000	+	+	+	−	−	−	*Escherichia coli.*
	1-3,000	+	+	+	+	−	−	
	1-4,000	+	+	+	+	+	+	
	1-500	−	−	−	−	−	−	
	1-1,000	+	−	−	−	−	−	
	1-1,500	+	+	+	+	+	−	
Phenol (preformed addition compound) [4]	1-730	−	−	−	−	−	−	
	1-1,460	+	+	+	+	−	−	
	1-2,190	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-4,000	+	−	−	−	−	−	
	1-6,000	+	+	−	−	−	−	
	1-8,000	+	+	+	−	−	−	
Phenol (1-1,000)	1-1-000	−	−	−	−	−	−	*Salmonella pullorum.*
	1-2,000	+	+	+	+	+	−	
Orthocresol (1-1,000)	1-2,000	+	−	−	−	−	−	*Salmonella suipestifer.*
	1-3,000	+	+	+	+	+	−	
	1-4,000	+	−	−	−	−	−	
	1-8,000	+	+	+	−	−	−	
	1-12,000	+	+	+	+	+	+	
Phenol (1-1,000)	1-2,000	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-4,000	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-2,000	−	−	−	−	−	−	
	1-4,000	+	+	+	−	−	−	
	1-6,000	+	+	+	+	+	+	

[1] Experiments conducted at 25° C.
[2] On basis of commercial dye; actual purity shown in table 1.
[3] Growth (+); no growth (−).
[4] The addition compound contains 68.5 percent of crystal violet and 31.5 percent of phenol.

Comparison of data in tables 1 and 2 indicates that the presence of phenol or orthocresol had little or no effect on the efficiency of triphenylmethane dyes against the Gram-positive *Staphylococcus aureus* but a varying favorable effect on their efficiency against Gram-negative organisms. The most notable effect was in the action of crystal violet on *Escherichia coli*. This increased efficiency is presumably due to the formation of addition compounds, described by Holmes and Hann,[2] who state, without presenting experimental data, that such compounds are germicidally active. In the case of crystal violet and phenol, the data in table 2 indicate that it was immaterial whether solutions of the two substances were merely mixed before use or whether the addition compound, containing 2 molecules of phenol to 1 molecule of crystal violet, was separated from admixed concentrated solutions, washed, and redissolved.

In table 3 are shown the effects, on the bactericidal powers of solutions of crystal violet, of the addition of sodium borate, sodium carbonate, or disodium phosphate. In this table, unlike tables 1 and 2, the dye concentration represents the actual dye content of the solution.

[2] HOLMES, W. C., and HANN, R. M. REACTIONS OF BASIC DYES WITH CYCLIC DERIVATIVES OF AN ACID CHARACTER. Stain Technol. 3:122-130. 1928.

TABLE 3.—*Influence of alkaline salts on the bactericidal efficiency of crystal violet* [1]

Alkaline salt added (1-1,000)	Dilution of dye [2]	Results after exposure for [3]— 5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	Test organism
None	1-1,000	+	−	−	−	−	−	*Eberthella typhi.*
	1-2,000	+	+	−	−	−	−	
	1-4,000	+	+	+	+	+	+	
Na_2CO_3	1-2,000	+	−	−	−	−	−	
	1-4,000	+	+	−	−	−	−	
	1-6,000	+	+	+	−	−	−	
None	1-200	+	+	+	−	−	−	*Escherichia coli.*
	1-400	+	+	+	+	+	−	
	1-1,000	+	+	−	−	−	−	
Na_2CO_3	1-2,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	−	−	
	1-6,000	+	+	+	+	+	−	
	1-8,000	+	+	+	+	+	+	
None	1-500	+	+	+	−	−	−	*Salmonella pullorum.*
	1-1,000	+	+	+	+	+	+	
	1-4,000	+	+	−	−	−	−	
Na_2CO_3	1,-8000	+	+	+	+	−	−	
	1-12,000	+	+	+	+	+	+	
None	1-400	+	−	−	−	−	−	*Salmonella suipestifer.*
	1-600	+	+	+	−	−	−	
	1-800	+	+	+	+	+	−	
Na_2CO_3	1-1,000	+	+	−	−	−	−	
	1-2,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	+	+	
Na_3HPO_4	1-500	+	+	−	−	−	−	
	1-1,000	+	+	+	+	−	−	
	1-2,000	+	+	+	+	+	+	
$Na_2B_4O_7$	1-500	+	+	−	−	−	−	
	1-1,000	+	+	+	+	−	−	
	1-2,000	+	+	+	+	+	+	
None	1-4,000	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-6,000	+	+	+	+	+	+	
Na_2CO_3	1-6,000	−	−	−	−	−	−	
	1-8,000	+	+	+	+	+	+	

[1] Experiments conducted at 25° C.
[2] On basis of true dye content; actual purity shown in table 1.
[3] Growth, (+); no growth, (−).

The results shown in table 3 indicate that the addition of sodium carbonate to solutions of crystal violet produced a marked increase in bactericidal efficiency, whereas sodium borate and disodium phosphate had comparatively little effect. The pH values of the mixtures containing sodium carbonate were slightly above 10, and of the mixtures containing sodium borate and disodium phosphate the pH values were between 8 and 9. When solutions containing sodium carbonate and crystal violet were allowed to stand several hours before being tested, some of the dye was precipitated and bactericidal efficiency diminished though still remaining greater than the efficiency of the dye alone.

EXPERIMENTS WITH CONTAMINATED HOG BLOOD

On the basis of the experiments with aqueous solutions, crystal violet was selected as the most promising dye for tests of bactericidal efficiency in the presence of a high concentration of defibrinated hog blood. Cultures of *Staphylococcus aureus*, *Salmonella suipestifer*, and *Escherichia coli* were mixed and added to the blood. After the addition of strong solutions of the bactericide, the mixtures were placed in an incubator at 37.5° C., and subcultures were made after 3, 7, and 14 days. Obviously, such tests are subject to considerable varia-

tion when attempts are made at duplication. Consequently, the data from a considerable number of experiments are only briefly summarized, as follows:

Mixtures containing phenol and crystal violet in concentrations of 1–500 and 1–2,000, respectively, and mixtures containing orthocresol and crystal violet in concentrations of 1–1,000 and 1–2,000, respectively, were sometimes effective and sometimes not. But mixtures containing orthocresol in a concentration of 1–500 and crystal violet in a concentration of 1–2,000 were uniformly effective even in the presence of very heavy bacterial contamination. Both phenol and orthocresol produced an undesirable thickening of the mixtures containing them. The admixture of sodium carbonate and crystal violet in final concentrations of 0.02 molar and 1–2,000, respectively, failed to kill the test organisms. Under like conditions, mixtures containing disodium phosphate were usually effective and mixtures containing sodium borate were uniformly effective. In these experiments the pH values of hog-blood mixtures containing sodium carbonate varied from 7.9 to 8.5; of those containing sodium borate, from 8.0 to 8.5; and of those containing disodium phosphate, from 7.3 to 7.4. Both sodium carbonate and sodium borate produced undesirable changes in the appearance and consistency of the mixtures, whereas disodium phosphate did not.

On the basis of the results obtained, the mixture of crystal violet and disodium phosphate was recommended as most suitable for the preparation of vaccine against hog cholera.

SUMMARY

Experimental information is presented regarding the bactericidal efficiency of several aniline dyes against *Eberthella typhi*, *Escherichia coli*, *Salmonella pullorum*, *Salmonella suipestifer*, and *Staphylococcus aureus*. Data presented indicate that the addition of phenol or orthocresol to solutions of crystal violet or methyl violet 2B greatly increases their bactericidal efficiency against Gram-negative bacteria. There is little or no increase in efficiency against Gram-positive bacteria. The addition of phenol or orthocresol to solutions of brilliant green or malachite green has little or no effect on their bactericidal efficiency.

The addition of sodium carbonate to aqueous solutions of crystal violet markedly increases bactericidal efficiency. The addition of sodium borate or disodium phosphate to such solutions produces little or no effect. In contaminated hog-blood mixtures kept at incubator temperature, crystal violet (1–2,000) with 0.02 molar sodium carbonate appears to be uniformly ineffective; with 0.02 molar disodium phosphate, usually effective; and with 0.02 molar sodium borate, uniformly effective, in killing the contaminating bacteria.

IDENTITY AND PARASITISM OF A SPECIES OF DOTHICHLOË [1]

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INTRODUCTION

Among the fungi that cause sterility diseases of certain grasses is an undescribed species of *Dothichloë* which has engaged the writer's attention intermittently for some 10 years. Its specific identity has not hitherto been distinguished, but in the literature it has been confused with related organisms so that an interest in the fungus itself is no less justifiable than in its rather remarkable pathological effects.

This fungus is rarely noted by the casual observer, although to one who has once become familiar with it the fructifications are evident first as white, then as black areas up to 2 cm in diameter on the upper (adaxial) leaf surfaces of infected grasses (pl. 1, *A* and *B*). The white or conidial stage is relatively conspicuous only under moist conditions when the grass leaves are distended, but the matured black ascostromata stand out plainly, being especially noticeable when, in response to drying, the partially inrolled leaf blades of the host plants fail to roll up at the normal rate in the stroma-bearing areas. Shortly after maturity, however, this black fungus layer sloughs off, restoring the leaf surface to a nearly normal coloration.

In a previous publication [3] on the life history of this fungus it was referred to as *Dothichloë atramentosa* Atk. Later studies, including comparisons with pertinent fungi exsiccati and reexamination of the literature, have verified the conclusions there recorded but have compelled the recognition of the fungus as a distinct species and have revealed considerable additional detail thought worthy of record.

GEOGRAPHIC DISTRIBUTION

In the southeastern part of the United States the fungus here discussed occurs commonly on *Sporobolus poiretii* (Roem. and Schult.) Hitchc. as a sterilizing parasite perhaps second in importance only to the ubiquitous *Helminthosporium ravenelii* Curt., and it has been found on at least nine other grass host species distributed among eight chiefly unrelated genera, not only in the Southeastern States but also in North Dakota and in Brazil, Surinam, and the West Indies.

[1] Received for publication November 4, 1938. Contribution No. 168 from the laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University. The material presented here is the result of work utilizing certain facilities provided by Harvard University in cooperation with the U. S. Department of Agriculture.

[2] The writer is indebted to J. L. Seal, Alabama Agricultural Experiment Station, and R. W. Davidson, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, for certain culture materials from Alabama and Louisiana, respectively; to E. F. Guba, Massachusetts Agricultural Experiment Station, for the use in 1930 and 1931 of greenhouse space at the Waltham Field Station; and particularly to W. H. Weston, professor of cryptogamic botany, Harvard University, for sustained interest and helpful criticism. To the many persons who have aided through the contribution of specimens it is impossible to express individual acknowledgment, but for permission to examine specimens in their charge and for incidental courtesies thanks are especially due to the following: F. J. Seaver, New York Botanical Garden; Erdman West, Florida Agricultural Experiment Station; and C. W. Dodge and D. H. Linder, Farlow Herbarium of Harvard University.

[3] DIEHL, WILLIAM W. CONIDIAL FRUCTIFICATIONS IN BALANSIA AND DOTHICHLOË. Jour. Agr. Research 41: 761–766, illus. 1930.

TAXONOMY

Although this organism already has a plethora of names, none of them is suitable because all are technically invalid. It seems necessary, therefore, to assign it a new name in order to prevent further confusion.

SYNONYMY

Dothichloë limitata n. sp.

Sphaeria epichloë Kze., 1827, nom. in sched. in Weigelt's exs. (not numbered), not *Botryosphaeria epichloë* Sacc., 1892, Fung. Herb. Brux. No. 11.

Dothidea atramentaria Berk. and Curt., 1876, nom. nud., Grevillea 4: 105; in Rav. F. Am. exs. No. 100, 1878; and in Ell. N. Am. F. No. 683, 1881.

Dothidea atramentaria Berk. and Curt. f. *eragrostidis-capillaris* Thüm., 1879, nom. nud., in DeThüm, Myc. Univ. No. 1068.

Ophiodothis vorax (Berk. and Curt.) Sacc. var. *atramentaria* Berk. and Curt., sec. Sacc., 1883, Syll. Fung. 2: 652.

Dothichloë atramentosa Atk., 1905, p. p. Jour. Mycol. 11: 260–261; not *Hypocrea atramentosa* Berk. and Curt., 1869, Cuban Fungi No. 758.

TECHNICAL DESCRIPTION

Hypostromate effuso, tenui, prosenchymatico, albido, in maturitatem nigro epiphyllo, usque 15μ in crassamentum, 200 mm in diam., raro in partibus minoribus diviso. Fructu imperfecto scolecosporo fugaci, in strato effuso ex hypothallo nascente; conidiophoris curtis, simplicibus, raro inflatis et ramosis; conidiis 18μ–30μ longis, 1μ–2μ latis. Fructu imperfecto amerosporo tantum in humore extremo super ascostromatem immaturem patefacto; conidiophoris simplicibus usque spatulatis, omne sterigmate apicali singulum conidium gerente; conidiis obovatis, usque 4μ longis, 3μ latis. Ascostromatibus effusis, maculiformibus, plerumque 10–15 mm in diam., rarius in pulvinulis divisis; superficie plana vel rugulosa, ex ostiolis leviter emersis punctata; contextu albido; peritheciis non arcte densis, ovatis vel lageniformibus, 180μ–315μ longis, 80μ–130μ latis, ostiolis curtis; muris peritheciorum 6μ–12μ crassis; ascis 105μ–160μ longis, 4μ–6μ latis; ascosporis 90μ–120μ longis, 1μ–1.5μ latis.

Hypostroma effuse, as a flat, thin, white prosenchymatous layer or film becoming black with age, on adaxial surfaces of unrolling leaves, rarely exceeding 15μ in thickness (vertical diameter) and 200 mm in horizontal diameter (regarding the leaf surface as the base), rarely as smaller separate units. Scolecosporous conidial fructification ephemeral, white to gray, covering the surface of the hypothallus with a palisade of conidia upon short, simple conidiophores, rarely swollen and branched; conidia 18μ to 30μ by 1μ to 2μ. (When conidia develop on small contiguous hypothalli the conidial fructifications coalesce to form a continuous hymenium.) Amerosporous conidial fructification developing under conditions of extreme moisture upon the immature ascostroma as a layer of simple to spatulate conidiophores, each with an apical sterigma bearing a single conidium; conidia obovate, up to 4μ by 3μ. Ascostromata uniformly effuse, maculiform, usually 10 to 15 mm in horizontal diameter, rarely exceeding 200μ in thickness, sometimes separated into pulvinuli by sterile areas; surface uniform to rugulose, black, punctate from the slightly emergent ostioles; context white; perithecia not crowded, ovate to lageniform, 180μ to 315μ by 80μ to 130μ, ostioles short; perithecial walls 6μ to 12μ thick; asci 105μ to 160μ by 4μ to 6μ; ascospores 90μ to 120μ by 1μ to 1.5μ.

Illustrations: Present paper, pl. 1; Chardon in Mycologia 13, pl. 14, fig. 12; and Diehl in Jour. Agr. Research 41, pl. 2, *D*, *E*, and fig. 3, *B*. The illustrations in the two last-named papers are incorrectly labeled "*Dothichloë atramentosa* Atk."

Distribution: On adaxial leaf surfaces of various grasses from North Dakota and Wisconsin to Florida, and in the West Indies, Mexico, Surinam, and Brazil.

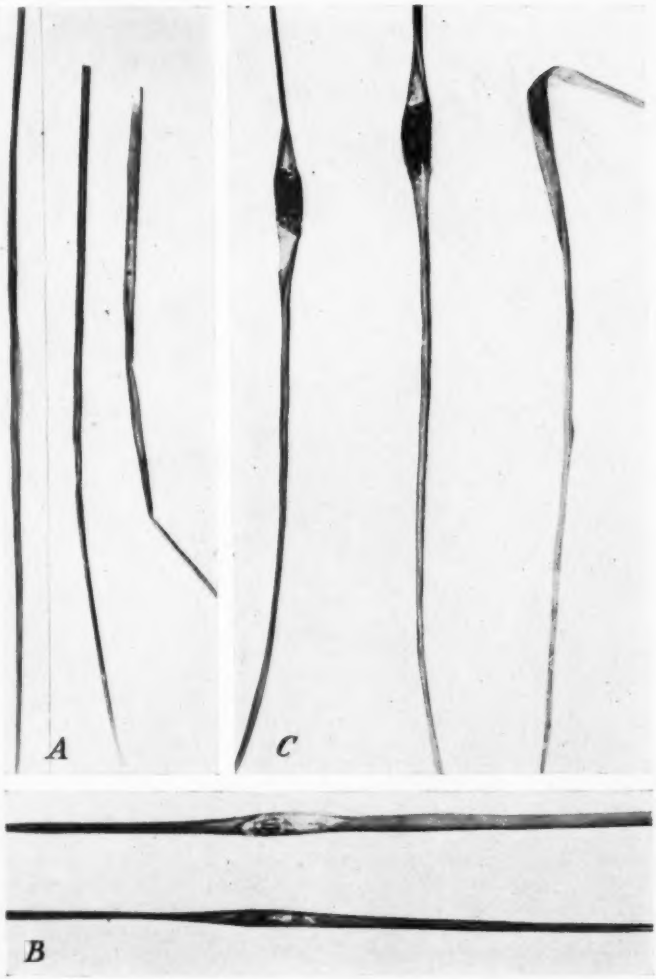
SPECIMENS CITED [4]

On *Calamagrostis canadensis* (Michx.) Beauv.—Wisconsin: 1912, J. J. Davis (N); 1928, J. J. Davis (W); 1919, C. Drechsler (W).

On *C. hyperborea* Lange.—North Dakota: 1922, Brenckle F. Dak. No. 532 (F, W), N. Dak. F. No. 1492 (W).

On *Chloris petraea* Swartz.—Cuba: 1911, Britton and Cowell No. 9899 (N, W). Puerto Rico: 1916, Whetzel and Olive No. 694 (N and in herb. J. A. Stevenson).

[4] Explanation of abbreviations used in citation of specimens: F=Farlow Herbarium, Cambridge, Mass.; Fl.=herbarium of the Florida Agricultural Experiment Station, Gainesville, Fla.; N=herbarium of the New York Botanical Gardens, New York; W=mycological collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.



Leaves of *Sporobolus poiretii* at different stages in the development of *Dothichloë limitata* (× 1): *A*, Unfolding leaves showing constricted region at the point of developing fructifications of *Dothichloë;* B, unfolded leaves showing expanded and rigid regions bearing conidia of the fungus; *C*, leaves bearing the black ascostromata of the fungus.

On *C.* sp.—Mexico: 1910, A. S. Hitchcock (W).
On *Ctenium aromaticum* (Walt.) Wood.—North Carolina: 1930, 1931, W. W. Diehl (F, W).
On *Eragrostis capillaris* (L.) Nees.—South Carolina: 1876, H. W. Ravenel in DeThüm. Myc. Univ. No. 1068 as *Dothidea atramentaria* B. and C. f. *eragrostidis capillaris* (F, W, N). (Paratype of *Dothichloë limitata.*) (The host here may very well be *E. refracta.*)
On *E. hirsuta* (Michx.) Nees.—Georgia: 1933, J. H. Miller (W).
On *E. refracta* (Muhl.) Scribn.—Georgia: 1931, W. W. Diehl (F, W). Florida: 1931, Weber, West., and Diehl (F, W). Virginia: 1932, W. W. Diehl (F, W).
On *E.* (?) sp.—South Carolina: ("Gramineae" of label) H. W. Ravenel No. 259 in Herb. Curtis (F); ('*Poa*' of label) Ravenel, F. Am. Exs. No. 100 (F, W, N); Ellis N. Am. F. No. 683 (F, W, N). Florida: 1897, R. Thaxter in herb. Thaxter (F).
On *Gymnopogon ambiguus* (Michx.) B. S. P.—Virginia: 1936, R. F. Smart (W).
On *Panicum* sp.—Mississippi; 1920, L. E. Miles 323 (W).
On *Panicum agrostoides* Spreng.—Missouri: 1906, H. L. Shantz (W).
On *Panicum* sp.—Mississippi: 1920, L. E. Miles No. 323 (W).
On *Sporobolus poiretii* (Roem. and Schult.) Hitchc.—Louisiana: 1931, R. W. Davidson (F, W). Mississippi: 1922, L. E. Miles No. 324 (W); 1930, R. J. Haskell (F, W). Alabama: 1898 (on "*Sporobolus radicans*" of label), G. W. Carver (Fl.); Auburn, July 17, 1929, J. L. Seal, type (F, W); 1931, J. L. Seal and W. W. Diehl (F, W). Georgia: 1908, H. A. Allard (W); 1930, 1931, J. B. Demaree (W); 1931 W. W. Diehl (F, W); 1932, J. E. Benedict, Jr. (F, W); 1933, Geo. Crisfield (F, W). Florida: 1928, G. F. Weber and ——— Nolan (Fl.); 1931, W. W. Diehl (F, W). Costa Rica: 1923, F. L. Stevens Nos. 10, 111, 151, 157 (W). Brazil: (Minas Geraes) 1929, Agnes Chase No. 10199½ (W).
On *Thrasya petrosa* (Trin.) Chase.—Brazil: 1930, A. Chase No. 11287 (W).
On Gramineae.—Surinam: 1827, Weigelt as *Sphaeria epichloë* Kze. (W).

IDENTITY OF SYNONYMS

It is possibly needful to explain here the particular reasons for proposing a new name to replace the early specific name "*epichloë*" and the later and much garbled "*atramentaria.*"

In the first place, it is questionable whether any of Weigelt's specific names possess validity unless published elsewhere than on the unnumbered specimens distributed by Kunze.[5] Any possible standing this specific name might have had is seriously brought into question by its particular validation by Saccardo,[6] who after studying the specimen of *Sphaeria epichloë* Kze. from the Royal Herbarium at Brussels described the material under the name *Botryosphaeria* (?) *epichloë* (Kze.) Sacc. He did not observe asci and noted spores "$7\mu \times 4\mu$," which he suggested might be conidia of *Dothiorella*; and it would seem to be a conidial fungus of questionable identity. Examination of the Weigelt specimen in the mycological collections of the Bureau of Plant Industry reveals it to be *Dothichloë limitata* without any evidence of contaminating *Dothiorella* or *Botryosphaeria.* It thus seems preferable to restrict Saccardo's name and description to some immature fungus not adequately described and not really significant in the nomenclature of the species under consideration, and to regard Kunze's name as invalid.

The name "*atramentaria*" used as a nomen nudum on the several fungi exsiccati cited above was apparently merely an error in spelling or printing for *atramentosa.* There is every reason to assume that Berkeley in 1876 [7] and Saccardo in 1883,[8] in publishing the name

[5] Specimens gathered by C. Weigelt in Surinam were named and distributed by Gustav Kunze in 1828 or thereabouts, bearing a printed label but lacking any numerical basis for citation. The particular printed label in question is as follows: "*Sphaeria* (periph. pulvin.) *epichloë* Kz. S. (epiphylla hypogena) oblonga gemella, atra: peritheciis ovalibus, ostiolo papillato, demum pertuso. In foliis gramineae, Gujan, Surinam, leg. et exsicc. Weigelt 1827. Determ. Kunze."
[6] SACCARDO, P. A. FUNGILLI ALIQUOT HERBARII REGNI BRUXELLENSIS. Compt. Rend. Soc. Roy. Bot. Belg. 31 (pt. 2): 224–258. 1892.
[7] BERKELEY, M. J. NOTICES OF NORTH AMERICAN FUNGI NO. 886. Grevillea 4: 105. 1876.
[8] SACCARDO, P. A. SYLLOGE FUNGORUM . . . v. 2, p. 814. 1883. Padova.

without a description, as well as Ravenel, DeThümen, and Ellis, in their use of the name on exsiccati, indicated no new species. Subsequent students of these two species have seemingly given this little or no concern. Atkinson [9] used the name *Hypocrea atramentosa* Berk. and Curt., of Berkeley's Cuban Fungi,[10] as the basis for *Dothichloë atramentosa* (Berk. and Curt.) Atk., although with apparent misgivings he included in his concept the species here called *D. limitata*. Atkinson clearly distinguished his species by its orientation upon lower leaf surfaces, but because of the "very thin stroma" expressed his doubt about the fungi exsiccati labeled "*Dothidea atramentaria*." Most curiously, he did not note the limitation of this "very thin stroma" to the upper or adaxial leaf surfaces. Both species have been grown by the writer contemporaneously, although upon different hosts, and their specific peculiarities in orientation have remained constant, i. e., *Dothichloë atramentosa* remained abaxial, and *D. limitata* adaxial. Differences in appearance and orientation might be explained as reactions owing to particular hosts were it not for the fact that, even when occurring on the same host species, the two fungi exhibit these differences in orientation. Both have been found and can be readily identified upon three grasses: *Chloris* sp., *Gymnopogon ambiguus*, and *Panicum agrostoides*.

PATHOLOGICAL CONSIDERATIONS

IDENTITY OF HOST SPECIES

The list of host names for specimens cited, included in the section on Taxonomy, shows a wide range of grass genera but does not pretend to comprise all susceptible species, because no extensive field studies have ever been undertaken. But where a specific name is noted there is reasonable certainty of identity. Through the kindness of Mrs. Agnes Chase and the late A. S. Hitchcock, reidentification or verification of particular specimens has been possible. The observer in the field is usually able to note the similarity of diseased (i. e. sterile but healthy-appearing) grass plants to nearby normal inflorescence-bearing specimens; verification of the then-assumed specific identity is readily made later in the laboratory by microscopic comparison of leaf sections.

FIELD OBSERVATIONS

Although adequate field studies have not been possible, it seems pertinent to note the localities and dates of field observations of diseased plants because they show some agreement with records of diseased plants cultivated under relatively continuous scrutiny in the greenhouse and garden. The following are the author's personal records of host, locality, month, and year for diseased plants noted, except that when later visits to the same place failed to detect the affected plants such dates are indicated by an asterisk (*):

On *Ctenium aromaticum:* Near Burgaw, N. C., April 1930, August* 1931, September 1931, December 1932, June 1933, September 1934, April 1938.

On *Eragrostis refracta:* Near Gainesville, Fla., September 1931; near Thomasville, Ga., September 1931; near Petersburg, Va., September 1932, September* 1933, October 1938.

[9] ATKINSON, G. F. THE GENERA BALANSIA AND DOTHICHLOË IN THE UNITED STATES WITH A CONSIDERATION OF THEIR ECONOMIC IMPORTANCE. Jour. Mycol. 11: 248-267, illus. 1905.

[10] BERKELEY, M. J. ON A COLLECTION OF FUNGI FROM CUBA. PART II., INCLUDING THOSE BELONGING TO THE FAMILIES GASTEROMYCETES, CONIOMYCETES, HYPHOMYCETES, PHYSOMYCETES, AND ASCOMYCETES. Jour. Linn. Soc. [London], Bot. 10: 341-392. 1869.

On *Sporobolus poiretii:* Auburn, Ala., September 1931; near Thomasville, Ga., 1931; Chattahoochee, Madison, and Gainesville, Fla., September 1931; Burgaw, N. C., April* 1931, September 1931, December* 1932, June 1933, September 1934, April* 1938.

At each time noted by an asterisk (*), considerable effort was made to find specimens, but without success. In these cases, however, the failure is explained by such local conditions as drought, destruction by fire or other causes, or by the dormant season. When growth was lush, the fungus was abundantly in evidence on certain plants. On these plants most leaves bore the fungus or showed certain chlorotic areas, which, as will be described later, are characteristic of dormancy or retardation of the fungus. With these slight exceptions, there was no difference between the color of diseased plants and that of healthy ones; diseased plants, even when abundant, are thus usually difficult to distinguish, because the mere absence of inflorescences in certain ones among thousands easily passes unnoticed. The limitation of infection to certain plants, on which practically every leaf bore the fungus while neighboring plants remained free from infection, suggested either marked differences in susceptibility of the several plants or else systemic infection. The latter hypothesis seemed the more reasonable in view of a lack of plants showing any intermediate condition. These observations were supplemented by the study of infected plants under garden and greenhouse culture where conditions permitted some degree of experimental control.

GARDEN AND GREENHOUSE CULTURES

Outdoor Transplantings

Infected plants (clumps) of three host species, namely, *Ctenium aromaticum*, *Sporobolus poiretii*, and *Eragrostis refracta*, have at various times been transplanted to a garden in Arlington, Va., and kept under observation particularly with regard to the succession of fungus fructifications and the possible development of normal inflorescences upon the plants. In each case the fungus-bearing leaf blades were removed, compelling the forcing of new leaves.

Table 1.—*Summary of garden transplantings of grasses infected with Dothichloë limitata*

Host	Origin	Time of transplanting	Time of last observation	Infected plants surviving
				Number
Ctenium	Burgaw, N. C.	April 1930	September 1930	5
		September 1931	October 1931	3
		April 1938	September 1938	2
Eragrostis	Gainesville, Fla.	September 1931	October 1931	4
	Petersburg, Va.	August 1932	October 1934	2
Sporobolus	Gainesville, Fla.	September 1931	October 1931	6
	Auburn, Ala.	July 1929	October 1929	6
		September 1931	October 1931	2
	Bogalusa, La.	July 1931	November 1932	4

During the months of June and July 1930, no observations were made, and during the season of June to September 1931, inclusive, 5 clumps of this origin were likewise grown in the open at the Harvard Botanical Garden, Cambridge, Mass., although inspected but once in August.

Except for the plantings made in September (table 1), in each case a healthy clump of a grass was planted contiguous to each diseased clump of the same species, so that the new leaves on the healthy plants might become infected upon coming in contact with the fungus on the leaves of the adjacent diseased plants. These healthy plants,

however, remained free of any infection during the entire time, whereas the newly forced leaves on the diseased transplants bore the fungus in regular succession until late autumn, when the newer leaves ceased to bear fungus fructifications.

Although healthy transplants of *Ctenium* remained sterile, those of *Eragrostis* in 1933 and 1934 and of *Sporobolus* in 1929 produced normal inflorescences. In contrast, none of the diseased plants developed any inflorescences or peduncles. In connection with the following studies of diseased plants under greenhouse conditions, additional observations on these outdoor plantings are compared with the results of the more controlled greenhouse cultures.

Greenhouse Cultures

In the autumn of 1930 the 3 above-noted infected grass species were removed to a greenhouse in the Harvard Botanical Garden at Cambridge, Mass., but only the *Ctenium* survived. There were 10 of these successful transplantings, in 10-inch pots. Since the greenhouse proved too cool in late autumn for rapid growth, 5 of the transplants were removed to a much warmer greenhouse available at the substation of the Massachusetts Agricultural Experiment Station at Waltham, where they were kept from late November through the month of February 1931, after which they were returned to the cooler Harvard greenhouse. In May 1931, when the Harvard greenhouse was warmer, the new *Ctenium* leaves again produced characteristic *Dothichloë* fructifications.

The Waltham greenhouse temperature averaged about 23° C., running considerably higher on sunny days but rarely dropping below 20° at night, so that the greenhouse at Waltham may be termed the warm greenhouse, while the one at Cambridge, where nighttime winter temperatures occasionally reached a minimum of 7°, may be termed the cool greenhouse. As a further check, two clumps transplanted to 6-inch pots were kept, from December 28 to April 6, under close observation in a Wardian case in the laboratory, where during that period the temperature normally ranged from 20° to 27°, with but seven instances of a 15° minimum.

During the entire time the plants were in the cool greenhouse, November to April, inclusive, there was very little vegetative growth of the *Ctenium* and no new appearance of the fungus. On the other hand, in the warm greenhouse the *Ctenium* plants grew new leaves at a rate comparable to summertime growth in the garden, and the fungus fructifications appeared on each leaf. Likewise, on the two plants kept in the Wardian case in the laboratory the fungus developed fruits upon the new leaves. Although in the cool greenhouse no new *Ctenium* leaves bore any fungus fructifications, they tended to show, but not uniformly, certain faintly chlorotic bands, occasionally with some constriction suggestive of the chlorotic areas where the fungus appeared normally. This same phenomenon was noted regularly during cool weather in the field and in garden plantings. Microscopic examination of such tissues, and of normal green-leaf tissues of infected plants as well, revealed the minute hyphae, 1μ to 2μ in diameter, characteristic of *Dothichloë* infection.

These observations on the behavior of the fungus in infected plants under low and normal temperatures indicate that low temperatures, although high enough for vegetative growth of both host and parasite, inhibit the fructifications of the fungus. It has been shown that a

5-month suppression of fructifications in the cool greenhouse had no inhibiting effect upon subsequent spore production after the infected grasses were again subjected to normal growing temperatures. The continued production of infected leaves without spore production through a long period also bore out the supposition that the infection was systemic.

In this connection it is noteworthy that in the field, as well as in garden and greenhouse culture, even under normally high-temperature conditions, infected plants of *Ctenium* occasionally bear some leaves that show neither chlorotic bands nor fungus fructifications, although microscopic examination of such leaves has revealed characteristic infecting hyphae.

INOCULATIONS

The presence on infected plants of seemingly uninfected leaves with or without slightly chlorotic bands was at first thought to be evidence that *Dothichloë limitata* caused localized disease areas rather than systemic infection. With that assumption, numerous attempts were made in greenhouses in Massachusetts, at Cambridge and at Waltham, during the winter season of 1930–31, and in garden culture at Arlington, Va., during the seasons of 1929, 1931, 1932, and 1934, to secure inoculation of emerging leaves on plants of *Ctenium aromaticum* and of 30 other possibly susceptible grasses, but without success. The methods used, in addition to the interplanting of healthy and diseased plants previously noted, were standard practices: Atomizer spraying and application of spores by both brush and fingertip rubbing, as well as needle injection by means of a hypodermic syringe, with germinable and germinating scolecosporous conidia and in part with ascospores. The complete failure to produce the disease by the inoculation of growing tissue in mature plants tended to discount the assumption of localized infection, yet it in nowise conflicts with the likelihood of systemic infection.

HOST SYMPTOMS AND SUPPRESSION OF FUNGUS FRUCTIFICATIONS

In greenhouse and garden, as in the field, newly forced leaves of infected plants tended to remain inrolled and at certain points on the blade failed to expand at the same rate as other leaves (pl. 1, *A*). At these points, on all hosts noted, there was a slight chlorosis, the constricted region being paler than the rest of the leaf. The whitish conidial fructifications were borne upon the adaxial leaf surfaces in the constricted region, maturing fully before this constricted region expanded (pl. 1, *A* and *B*). When these spore-bearing surfaces were fully expanded they became rigid and were chiefly noticeable because apparently unable to roll up to the same extent as the rest of the leaf on drying. In most cases, after the conidial fructifications reached maturity they were succeeded in from 1 to 2 weeks by black ascostromata (pl. 1, *C*) bearing perithecia and ripe ascospores. These ascostromata disintegrated shortly after reaching maturity. After the rather evanescent ascostromata disappeared, the stiffened leaf areas remained rigid and slightly chlorotic, tending to form a conspicuously expanded area on a normally inrolling recurving leaf. These leaves never seemed otherwise to suffer any immediate disadvantage, because they remained a nearly normal green color long after the stromata had disappeared. In all cases under observation, however, individual plants so infected have never shown any tendency to produce

inflorescence. This coincides with field records in that inflorescences and seed production appeared to be absent uniformly from plants infected with *Dothichloë limitata*.

In garden culture of infected plants it has been noted consistently that on the newer leaves produced in late autumn no fungus fructifications developed, the leaves being seemingly free of infection. But in *Ctenium* there were slightly chlorotic leaf areas comparable in size and position to fungus-bearing areas, although they remained sterile. Microscopic examination, however, always revealed the characteristic mycelium 1μ to 2μ in diameter, in such tissues.[11] This chlorotic tendency was less noticeable in *Sporobolus* and in *Eragrostis* than in *Ctenium*.

DISCUSSION

The host plants thus far recorded for *Dothichloë limitata* are widely distributed geographically, although except for the two species of *Calamagrostis* they are not of economic importance. It is, however, of significance that when infected by this fungus these various grasses are sterilized by it. This condition of sterility generally passes unnoticed because it lacks the conspicuous chlorotic effects or obvious lesions usually associated with disease. The parasite, which sterilizes the host through preventing the formation of any semblance of an inflorescence but which produces no other readily recognizable effects, presents a role that has been little considered in phytopathological literature. It it is not convenient here to attempt an evaluation of the possible factors affecting this sterility phenomenon, but such incidental observations as those upon the effect of low temperature in preventing sporulation of the parasite may well aid in explaining its predominantly southern range. In more northern regions the fungus may be present in grass tissues without sporulation and therefore without observational record. In those cases its presence could be detected only by microscopic examination. The presence of minute hyphae in seemingly healthy grass tissues has long been known, particularly in the case of various inconsistent records of observations and discussions of "the *Lolium* fungus." *Dothichloë limitata* is doubtless merely one of a number of similar intramatrical parasites of grasses and sedges that sterilize their host plants without producing any conspicuous lesions or chlorosis.

SUMMARY

Dothichloë limitata, here described as new, is distinguished from a closely related fungus by its morphology and its habits.

Its known distribution, from Brazil through the southeastern part of the United States, with a host range comprising species of eight different grass genera, is based on an extensive herbarium record.

Observations of infected grasses in field and greenhouse indicate that infection is systemic.

It is shown that low temperatures inhibit the fructifications of the fungus but do not eliminate the infection.

Infected plants, except for a failure to produce inflorescences, appear superficially healthy because of a normal coloration.

[11] Such hyphae are demonstratable by crushing and macerating the mycelium under a cover glass and then staining it with erythrosin (phloxine).

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